# Cognitive Function and Symptoms During Hypoglycaemia and Hyperglycaemia

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# Declaration

(a) This thesis was composed by me.

(b) I made a substantial contribution to all studies described here:

**Study 1**
This was performed and written primarily by me.
The study design was contributed to by Drs Andrew Sommerfield and Kate Allen of the Department of Diabetes, Royal Infirmary of Edinburgh, and Andrea Greve of the Department of Informatics, University of Edinburgh.
Analysis of brain imaging data was performed by Adam McNamara, Enrico Simonotto and Martin Meyer of the Centre for Functional Imaging Studies, University of Edinburgh. Analysis of other data was performed by me.

**Study 2**
This was designed, analysed and written primarily by me.
The experimental procedures were shared equally between me and Dr Nicola Zammitt of the Department of Diabetes, Royal Infirmary of Edinburgh.

**Study 3**
This was performed, analysed and written primarily by me.

**Study 4**
This was designed, performed, analysed and written primarily by me.

(c) This thesis has not been submitted for any other degree or professional qualification.

Roderick Edward Warren

Date: 18/12/08

# Thanks

I am absolutely indebted to Professor Brian Frier, without whom this thesis and the research it describes would not exist. His contribution in terms of clinical, research and editorial expertise cannot be overstated. Even more important has been his continuous support, and an enthusiasm for research that has kindled the same in me, as it has numerous researchers before and after.

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# Dedication

For Emma.

## THE UNIVERSITY OF EDINBURGH

# ABSTRACT OF THESIS


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| *Candidate Name* | Roderick Edward Warren |
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In the first chapter, the definitions, frequency, causes and consequences of clinical hypoglycaemia are discussed. Previous research on the cognitive effects of acute hypoglycaemia is reviewed in the second chapter. Four studies of the effects of blood glucose manipulation on cognitive function are then described.

In Study 1, the effect of acute hypoglycaemia on memory consolidation was studied in non-diabetic subjects. Functional brain imaging was used to identify regions with altered metabolism following hypoglycaemia. No effect of hypoglycaemia was seen on memory or brain imaging results, suggesting that acute hypoglycaemia does not affect previously-formed memories, though other possibilities are discussed.

In study 2, the effects of acute hypoglycaemia on learning and recall were studied, and a novel test of prospective memory, mimicking memory in real life, was developed. Subjects had type 1 diabetes and either normal or impaired awareness of hypoglycaemia. Both learning and recall were impaired during hypoglycaemia. Prospective memory was impaired to a similar degree, reinforcing the ecological validity of laboratory cognitive function testing. No statistically significant differences between normal- and impaired-awareness subjects were seen, but this may have been due to lack of statistical power.

A previous study had unexpectedly reported that high-level cognitive functions were unaffected by hypoglycaemia. Study 3 re-addressed this issue using a more difficult cognitive test to exclude the possibility of a ceiling effect, and impairment by hypoglycaemia was confirmed. A separate task, intended to indicate which of speed and accuracy of cognitive processing is primarily affected, yielded no significant results.

In study 4, the symptoms of hyperglycaemia reported by 400 people with insulin-treated diabetes were examined using factor analysis. Four categories were identified, and labelled as 'osmotic', 'neurological', 'mental agitation' and 'malaise'. Substantial overlap with hypoglycaemia symptoms was noted. The mean blood glucose level for symptom onset was 15 mmol/l, with a tendency for less intense symptoms and a higher symptom threshold in older people and those with impaired hypoglycaemia awareness.

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# Chapter 1

# Clinical and Biochemical Aspects of Hypoglycaemia

## 1.1 Introduction

In type 1 diabetes there is total, or near-total, loss of insulin secretory function by the pancreas; a principal feature of the condition is an elevated blood glucose level, or hyperglycaemia. Insulin was isolated and used as therapy for type 1 diabetes in the 1920s by the Toronto pioneers Frederick Banting, Charles Best, James Collip and John Macleod. Insulin was confirmed to lower the blood glucose level, but in large doses it caused a new phenomenon: low blood glucose, or hypoglycaemia. Rabbits injected with large doses of insulin were seen to become wildly hungry, then comatose, and then to die. Similar (but less drastic) side effects were seen when the new substance was first used in humans [1]. Insulin therapy has undergone numerous refinements, both revolutionary and incremental, since then, which have permitted more precise control of blood glucose levels. Despite these advances, hypoglycaemia remains a common and feared side effect [2,3,4]. At best, hypoglycaemia causes unpleasant symptoms that require the person with diabetes to treat themselves to correct the condition. At worst, hypoglycaemia can cause confusion or collapse, creating a risk of physical injury, especially during potentially risky activities such as driving. More pervasively, the fear of possible hypoglycaemia can engender a state of perpetual anxiety that may be a barrier to a normal working or domestic life, and a barrier to achievement of the good glycaemic control that is needed to prevent diabetes-related complications.

In part, hypoglycaemia persists as a clinical problem because the targets for glycaemic control are continually being adjusted. The Diabetes Control and Complications Trial showed that lowering blood glucose with intensive insulin therapy reduces the incidence and progression of microvascular and macrovascular diabetes complications, at the

expense of an increased frequency of hypoglycaemia [5,6]. Advances in insulin therapy and monitoring have permitted people with diabetes to achieve more physiological insulin profiles. These improvements might be used to achieve a reduction in hypoglycaemia for a similar level of glycaemic control, but it is arguably more important that glycaemic control should improve for a similar (or slightly reduced) frequency of hypoglycaemia. Thus, until insulin therapy is perfected (in the sense that blood glucose can be contained entirely within the non-diabetic range), further improvements in insulin therapy will not eradicate hypoglycaemia but will continue to offer the opportunity to diminish the burden of either hypoglycaemia or hyperglycaemia.

It is hard to overstate the importance of hypoglycaemia in insulin-treated diabetes. Were it not for the fact that an excess of insulin causes hypoglycaemia, it would be possible to avoid all the complications of diabetes by simply increasing doses of insulin to abolish hyperglycaemia. Since hypoglycaemia appears to be a significant but necessary evil in the management of diabetes mellitus with insulin, it is important that we should understand its causes and effects. An enormous body of research has developed on the topic, some of which is discussed in this chapter and the next.

## 1.2 Definitions of Hypoglycaemia

Although the definition of hypoglycaemia as 'a low blood glucose level' is simple, the type of blood in which glucose is measured is important. Arterial blood supplies glucose to tissues, and so the arterial plasma glucose concentration is the gold standard measurement. However, arterial sampling is invasive and can be painful, so venous or capillary measurements are more often used in clinical practice. It is important to remember that under conditions such as cold (when peripheral blood flow is reduced) or vigorous exercise (when peripheral glucose uptake is increased), venous or capillary glucose measurement may be a poor index of 'glycaemia' [7]. Also, clinical laboratory measurements are in plasma glucose, to remove the confounding effect of a variable haematocrit. Near-patient measurements and measurements in many research studies are in whole blood, which typically yields results that are 10-20% lower than the equivalent plasma glucose concentration [8].

### 1.2.1 Biochemical definitions by population norms

In theory, a biochemical definition of hypoglycaemia may be derived by reference to the normal range of blood glucose in healthy people. Service, in a generally excellent review article, stated that healthy people will usually maintain venous plasma glucose between 3.3 and 5.6 mmol/l, except in the immediate post-prandial period. He also stated that "normal persons may have plasma glucose levels well below 50 mg per deciliter [2.8 mmol/l] while fasting" and recommended that a plasma glucose below 45 mg/dl (2.5 mmol/l) be required to diagnose a hypoglycaemic disorder [9].

Closer consideration of the issue shows that it is impossible to create a single biochemical definition of hypoglycaemia. It is not uncommon for apparently healthy people to experience frequent symptoms suggestive of a low blood glucose level, often shortly after eating a meal. In earlier decades, 'functional' or 'post-prandial' hypoglycaemia had been fashionable diagnoses, the investigation of which had occupied considerable resources for little appreciable benefit [10,11]. Service appears to have been promoting a pragmatic approach, so that a disease label is attached only to conditions that without treatment are likely to progress to cause other, more serious, morbidities – such as insulinomas, liver disease, adrenal failure and metabolic storage

disorders. However, the same evidence showing that profoundly low blood glucose levels are very rare in 'functional hypoglycaemia' also shows both that symptoms are more common when capillary blood glucose is below 3.3 mmol/l, and that blood glucose levels below 3.3 mmol/l are more common in people with symptoms [12]. More prosaically, personal experience relates that almost everyone experiences milder versions of the classical symptoms of hypoglycaemia after exercise or fasting. As will be discussed later, these symptoms prompt the ingestion of food, and are part of the body's homeostatic mechanisms for glucose. The fact that very low glucose levels may be seen in healthy people during a prolonged period of intentional food avoidance (e.g. the 72-hour fast) does not mean that these are 'normal'.

Thus, the population does not categorize neatly into 'unhealthy' and 'healthy' people who do and do not suffer hypoglycaemia, and a biochemical definition based on the population distribution of blood glucose levels is unsatisfactory. Hypoglycaemia is better defined with reference to its effects.

### 1.2.2 Biochemical definitions of hypoglycaemia by its effects

Progressive, experimentally-induced hypoglycaemia produces a hierarchy of responses which are summarized in Figure 1.1 (derived from Schwartz *et al* [13] and Mitrakou *et al* [14]). The earliest response is the release of epinephrine, glucagon and growth hormone, which tend to slow or reverse the fall in blood glucose. This counterregulatory response begins at an arterial blood glucose level of approximately 3.8 mmol/l, and may be taken as evidence that the body 'perceives' an abnormality.

Alternatively, the onset of symptoms at around 3.0 mmol/l may be considered more important. However, other conditions such as anxiety or alcohol intoxication may produce the same symptoms or behaviours. A definition of hypoglycaemia based on symptoms alone would be extremely unreliable, and such definitions are only rarely used in clinical studies and never in laboratory studies.

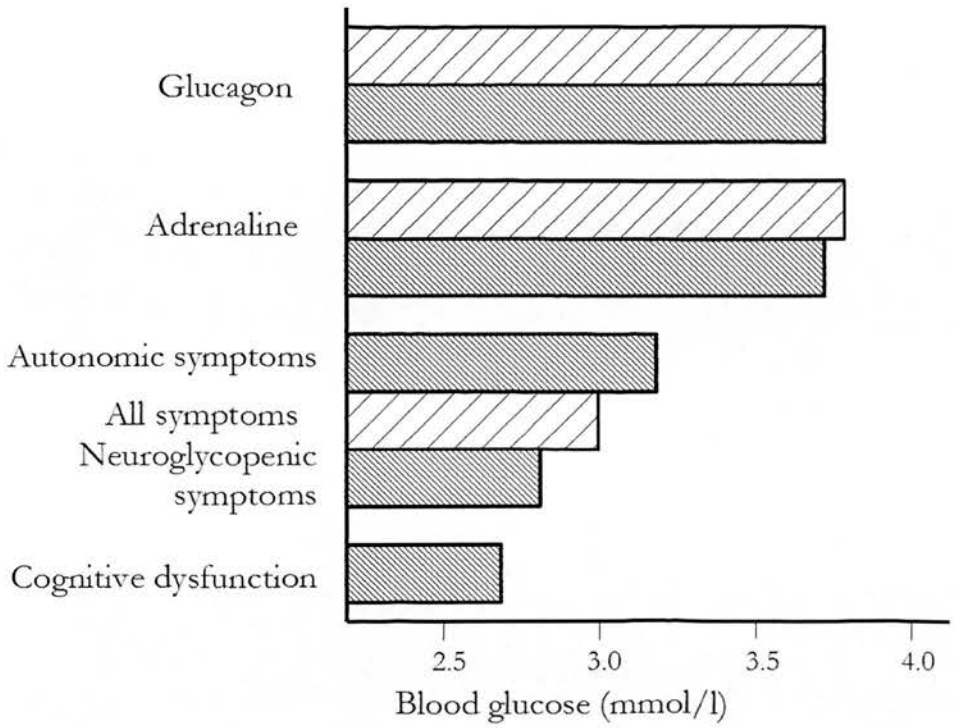


**Figure 1.1.** Glycaemic thresholds for initiation of various hypoglycaemic responses. Based on data from Schwartz *et al* [13] (light bars) and Mitrakou *et al* [14] (dark bars).

An important consideration is that the level of blood glucose at which a statistically significant change is first detected is not necessarily the level at which the hypoglycaemic response begins. Mild hypoglycaemia may cause only a small increase in symptom scores which fails to achieve statistical significance. In both Schwartz *et al* [13] and Mitrakou *et al* [14], 10 subjects were studied, which may not provide much statistical power. Thus, it is more accurate to say that the evidence shows that hormonal counterregulation begins somewhere above, but close to, 3.8 mmol/l, and that symptoms begin somewhere above, but close to, 3.0 mmol/l. This appears to be more compatible with anecdotal reports of simple hunger and tiredness at blood glucose levels between 3 and 4 mmol/l in both non-diabetic people and people with diabetes.

In diabetes, as will be discussed later, glycaemic thresholds for counterregulation, symptoms and cognitive function appear adapt to prevailing blood glucose levels (which may be very high or very low) in diabetes. Thus, it is possible for a person with diabetes

to have a blood glucose level of 2.5 mmol/l and to feel normal, with no detectable cognitive impairment or counterregulatory activity. However, it is sensible to consider these episodes as hypoglycaemia, since the adaptation is itself a consequence of a metabolic disorder and, as will also be discussed later, is not entirely benign.

People with diabetes with chronically elevated blood glucose levels may experience symptoms of low blood glucose at blood glucose levels above 4.0 mmol/l. As will be discussed later, this acute state is probably benign (though the chronic hyperglycaemia is not), but it is clinically relevant because the sensation of hypoglycaemia affects the person's management of their diabetes.

### 1.2.3 Definition of severity of hypoglycaemia

It is almost universally accepted that the most useful distinction in terms of severity is between those episodes of hypoglycaemia that are self-treated, and those for which assistance is required. Thus, 'mild' hypoglycaemia is self-treated and 'severe' hypoglycaemia requires third party assistance.

This definition remains a little subjective, as whether assistance is given depends on whether it is available, and on the beliefs and attitudes of the victim and any third parties. It is possible that a person with diabetes may eventually, and with difficulty, self-treat an episode of hypoglycaemia (labelled mild) for which they would have wished assistance had they been in company (labelled severe). The nature of assistance given (e.g. encouragement to eat, buccal glucose administration or parenteral therapy) is highly dependent upon individual circumstances, and therefore not a guide to severity.

### 1.2.4 Conclusions regarding hypoglycaemia definitions

There can be no single definition of hypoglycaemia. One reasonable biochemical definition would be a blood glucose level below 3.0 mmol/l, in the sense that it in healthy people it rarely occurs, and when induced experimentally is associated with symptoms, hormonal responses and adverse effects on cognition. This definition is specific but not sensitive: it may misclassify milder episodes that nonetheless cause

recognisable symptoms, and if caused by insulin therapy, may progress to cause harm if left untreated.

A reasonable clinical definition would be a modification of Whipple's triad: a combination of typical symptoms, a blood glucose low that is subjectively low for that person, and relief of symptoms when blood glucose is raised [15]. However, this definition fails to include asymptomatic hypoglycaemia in persons with impaired awareness of hypoglycaemia. Such episodes are important, as they can be markers for a condition that is not benign (see sections 1.6 and 2.5).

In clinical practice, people with diabetes are often advised to consider a blood glucose level below 4.0 mmol/l as hypoglycaemic, and Diabetes UK (the principal charity for people with diabetes in this country) make this recommendation [16]. It includes a sensible margin of safety, allowing for the possibility that the blood glucose level may be falling, for measurement error and for individual differences in glycaemic thresholds.

## 1.3 Frequency of hypoglycaemia in insulin-treated diabetes

### 1.3.1 Frequency of hypoglycaemia and glycaemic control

Insulin-treated diabetes is characterized by erratic rises and falls in blood glucose. Not every fall will result in blood glucose dipping into the hypoglycaemic range; the frequency with which this occurs is also dependent on the prevailing level of blood glucose. This is illustrated in Figure 1.2, which shows the same hypothetical glucose excursion at two levels of glycaemic control. It is apparent that the frequency of hypoglycaemia will be increased in people with diabetes who achieve low average blood glucose levels ('strict glycaemic control').

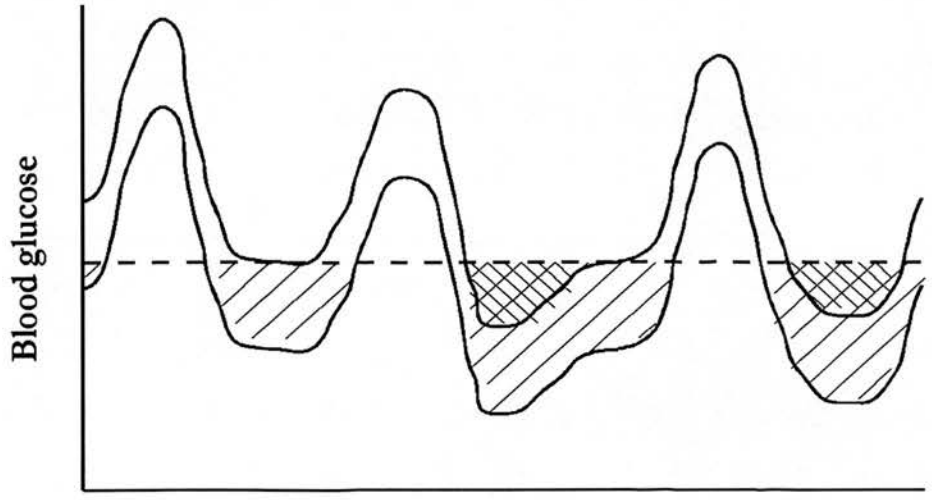


**Figure 1.2**. The same glucose excursion results in more hypoglycaemia when glycaemic control is strict (diagonal hatching) than when glycaemic control is relaxed (cross hatching).

This is borne out in results from surveys and clinical trials. Frequency of hypoglycaemia increased as median blood glucose concentration decreased in a study by Thorsteinsson *et al* [17]. The frequency of mild and severe hypoglycaemia was increased roughly two- to threefold in patients with type 1 diabetes allocated to intensive glycaemic control in the Diabetes Control and Complications Trial [5]. In the United Kingdom Prospective Diabetes Study of type 2 diabetes, both minor and major hypoglycaemic episodes

occurred approximately twice as often in the intensively-treated group as in the conventionally-treated group (based on intention-to-treat analysis; there was considerable crossover between treatment types within groups) [18]. Thus, estimates of hypoglycaemia frequency in diabetic patients as a whole conceal a wide individual variation in rates of hypoglycaemia.

### 1.3.2 Frequency of mild hypoglycaemia

The average frequency of mild hypoglycaemia in type 1 diabetes has been reported as about once to twice per week [3,4]. However, this probably underestimates the rate of biochemical hypoglycaemia, which can only be detected if symptoms are generated and identified, or by chance on routine testing. The use of continuous glucose monitoring systems (CGMS) has demonstrated that asymptomatic hypoglycaemia is extremely common. Bode *et al* reported an average of 2.1 episodes of biochemical hypoglycaemia (defined as blood glucose <3.9 mmol/l) per day in patients with type 1 diabetes, with patients being hypoglycaemic for 8% of the monitoring period [19]. Chico *et al* also detected frequent asymptomatic events with CGMS [20], and in both studies hypoglycaemia was especially frequent during the night. These may be overestimates, as CGMS devices have only been shown to have good accuracy when a more stringent definition of hypoglycaemia is used (< 2.2 mmol/l), but the frequency of asymptomatic nocturnal hypoglycaemia has also been clearly demonstrated in earlier laboratory-based studies [21,22].

In type 2 diabetes, frequency estimates are lower overall, but more variable, ranging from approximately five to 30 episodes per person per year. This is likely to reflect the different treatment options (oral medication or insulin) and the progression of the condition from milder insulin resistance or deficiency to an increasingly insulin-deficient state resembling type 1 diabetes [23].

### 1.3.3 Frequency of severe hypoglycaemia

In population surveys, estimates of the incidence of severe hypoglycaemia in type 1 diabetes range from 1.0 to 1.7 episodes per person per year, with annual prevalences of 30 to 40% [4,24,25,26]. The Diabetes Control and Complications Trial reported lower

frequencies of 0.19 (conventional treatment group) and 0.62 (intensive) episodes per year, which must be at least partly due to the highly selected patient group [5]. Patients with two or more episodes of severe hypoglycaemia in the preceding two years were excluded from the DCCT, based on a pilot feasibility study which had shown that such patients were at high risk for recurrent severe hypoglycaemia with intensive treatment. For type 2 diabetes, population surveys have reported incidences ranging between 0.07 and 0.73 episodes per year per person [23]. The incidence is again lower in clinical trials: the United Kingdom Prospective Diabetes Study reported incidence rates of approximately 0.02 episodes per person per year in the intensively-treated group [18].

## 1.4 Causes of hypoglycaemia in insulin-treated diabetes

In diabetes, all episodes of hypoglycaemia result from a mismatch between insulin activity and insulin requirement. This is often considered to mean the balance between insulin dosing and food intake. Failure to reduce insulin doses when meals are reduced or omitted is probably to blame for many episodes of hypoglycaemia, but there are many other factors which affect the balance between insulin activity and requirement. These may be divided into those which occur daily as a result of inherent difficulties with subcutaneous insulin, and those which arise in special circumstances.

### 1.4.1 Common causes of hypoglycaemia

*Insulin activity profile.* In non-diabetic humans, the rise in blood insulin levels following a standard meal lasts in the region of 2 hours [27]. By contrast, soluble human insulin (e.g. Actrapid, Humulin S) given by subcutaneous injection peaks at 2-4 hours and has a total duration of action of 8-10 hours. The modern insulin analogues lispro and aspart peak earlier at 1-2 hours and have a shorter duration of action of around 5-8 hours [28,29], although they still do not quite match non-diabetic post-prandial insulin secretion. A dose of insulin that is adequate to control post-prandial hyperglycaemia will therefore tend to cause hypoglycaemia around 3-4 hours after a meal. In practice, people with insulin-treated diabetes must choose insulin doses that are a compromise between control of hyperglycaemia and avoidance of hypoglycaemia. Similar problems exist with the longer-acting isophane, lente and ultralente preparations of human insulin, which are often used to provide overnight insulin, but peak around 2-6 hours after injection and therefore tend to cause nocturnal hypoglycaemia.

*Insulin absorption.* Under laboratory conditions, the coefficient of variation for the activity of a given dose of insulin may be as high as 60% [30]. In other words, the same dose of insulin given on different days may have markedly different effects on blood glucose, even when other factors are controlled.

*Infrequent blood glucose testing.* Many people with insulin-treated diabetes find blood glucose measurement uncomfortable and inconvenient, and do not measure blood glucose frequently. However, estimation of blood glucose levels on the basis of

symptoms is unreliable [31,32,33]. The effects of recurrent hypoglycaemia on symptom generation will be discussed later in section 1.6.

*Meal constitution.* For a given amount of total carbohydrate, a starchy meal will release monosaccharides more slowly than a meal containing simple sugars, and thus will cause a smaller but more prolonged rise in blood glucose levels. This is reflected in the concept of glycaemic index (GI). The rate of absorption of a meal also varies with its fat and liquid content [34,35].

### 1.4.2 Special circumstances that may contribute to hypoglycaemia

*Exercise.* Glucose is utilized by muscles during exercise, independent of circulating insulin levels, and hypoglycaemia may occur during or following exercise if appropriate corrective action is not taken. Less obviously, there is a delayed risk of hypoglycaemia after exercise has ceased; this may last up to 24 hours, with nocturnal hypoglycaemia being particularly likely without corrective action [36].

*Stress.* Both physical and emotional stress can cause release of adrenaline, cortisol and other hormones that tend to cause hyperglycaemia. This leads to a fluctuating increase in insulin requirements, with erratic blood glucose levels as a result, and hence an increased risk of hypoglycaemia.

*Gastroparesis.* Abnormal gastro-intestinal motility, believed to be a consequence of autonomic neuropathy, results in delayed and less predictable absorption of food following meals [37].

*Illness.* Illness may cause hypoglycaemia through reduced enteric carbohydrate absorption (anorexia, vomiting, and malabsorptive disorders such as coeliac disease). Hypoadrenalism predisposes to hypoglycaemia. Renal failure reduces insulin clearance and hence increases insulin activity for a given dose.

*Drugs and toxins.* Alcohol suppresses hepatic glucose output, with the result that the risk of nocturnal hypoglycaemia is increased following evening alcohol intake. Some drugs, including ACE inhibitors and disopyramide, appear to increase insulin sensitivity [38,39]. Withdrawal of drugs that cause insulin resistance (e.g. systemic corticosteroids) also results in a relative increase in insulin sensitivity.

## 1.5 Effects of hypoglycaemia

At the head of any such list should be the potential effects of hypoglycaemia on the home, educational and working life, and emotional wellbeing of the person with diabetes. These psychosocial aspects of hypoglycaemia have been reviewed extensively elsewhere, but are outwith the scope of this thesis, the subjects of which are primarily the symptoms and cognitive effects of altered glucose metabolism.

### 1.5.1 Hormonal responses to hypoglycaemia

The effects of different levels of hypoglycaemia in healthy humans have been defined by experiments in which progressive hypoglycaemia was induced. These have been summarized in Figure 1.1, and begin with the release of counterregulatory hormones, including glucagon, adrenaline, cortisol and growth hormone at blood glucose levels of approximately 3.8 mmol/l. The hormonal responses are counterregulatory, triggering a corrective rise in blood glucose.

### 1.5.2 Symptom responses to hypoglycaemia

The symptoms of hypoglycaemia begin at, or a little above, a blood glucose level of 3.0 mmol/l (also summarized in Figure 1.1). The symptoms of hypoglycaemia have been studied using factor analysis, a statistical technique that identifies separate groups of symptoms that correlate strongly [40,41]. The value of this approach rests on the probability that symptoms that occur together are caused by the same underlying physiological process. The two main groups have been interpreted as representing the autonomic (or adrenergic) effects of hypoglycaemia, and neuroglycopenia (confusion and other manifestations of cerebral dysfunction) (Table 1.1). Symptoms attributed to the hormonal and adrenergic nervous system reactions include tremor, sweating, increases in heart rate and reduced salivation. Symptoms attributable to tissue glycopenia (specifically affecting the brain, i.e. neuroglycopenia) include confusion, loss of coordination and drowsiness.

Of the symptoms in Table 1.1, hunger can be considered one of the counterregulatory mechanisms because it triggers corrective behaviour (ingestion of food). In factor analysis, hunger co-segregated with the autonomic symptoms, although other evidence indicates that hunger is generated in brain regions that are directly triggered by other glucose-sensing brain region(s) rather than via adrenergic mediators [42,43]. It should be noted that applying factor analysis to symptom scores will identify symptoms that tend to occur together, rather than necessarily sharing the same physiological cause. The 'autonomic' symptoms show significant adaptation to recurrent hypoglycaemia, whereas cognitive adaptation appears to occur to a limited extent only (discussed in more detail in sections 1.6 and 2.5). An alternative interpretation of the factors in Table 1.1 is: (1) typical symptoms triggered by cerebral glucose sensing – may become significantly diminished or lost; (2) typical symptoms directly due to cerebral glucose privation – may adapt a little; (3) non-specific physical symptoms.

| ***Factor 1*** *'Autonomic'* *(Glucose sensing?)* | ***Factor 2*** *'Neuroglycopenic'* | ***Factor 3*** *'Malaise'* |
|---|---|---|
| Sweating | Confusion | |
| Palpitation | Drowsiness | Nausea |
| Shaking | Odd behaviour | Headache |
| Hunger | Speech difficulty | |
| | Incoordination | |

**Table 1.1.** Symptoms of hypoglycaemia grouped by factor analysis. Based on [40,41].

### 1.5.3 Cognitive effects of hypoglycaemia

Measurable cognitive dysfunction may begin at a slightly lower blood glucose level (around 2.8 mmol/l in non-diabetic humans) than is required for the onset of symptoms. Subsequently more severe cognitive dysfunction develops, and loss of consciousness, convulsion, coma, and ultimately brain damage and death occur as blood glucose levels fall below about 1.5 mmol/l. Cognition during hypoglycaemia is discussed in detail in Chapter 2.

## 1.6 Adaptation to hypoglycaemia and impaired awareness of hypoglycaemia

In insulin-treated diabetes of long duration, a lower blood glucose level may be required for generation of hypoglycaemic symptoms, and the intensity of symptoms felt at a given level of hypoglycaemia may be diminished. This phenomenon has been referred to as impaired hypoglycaemia awareness; 'hypoglycaemia unawareness' is less appropriate, as it is rare that no symptoms are experienced at all. When a person with diabetes has diminished warning symptoms for hypoglycaemia, their ability to take appropriate action to avoid severe hypoglycaemia is limited. The frequency of severe hypoglycaemia has been shown to be increased in patients who report impaired hypoglycaemia awareness [44,45,46].

Experimental research has demonstrated that counterregulatory failure is associated with impaired hypoglycaemia awareness. As detailed above in section 1.5, release of hormones including glucagon, adrenaline (epinephrine), cortisol and growth hormone occurs in the early stages of hypoglycaemia in non-diabetic humans. All of these hormones have a hyperglycaemic effect, though glucagon and adrenaline are the most potent. In type 1 diabetes, the adrenaline (epinephrine) response to hypoglycaemia becomes attenuated, although does not disappear entirely [47,48]. The glucagon response is diminished early in the course of type 1 diabetes, and within a few years becomes negligible or non-existent. It has long been known that patients who do not mount a glucagon response to hypoglycaemia can secrete glucagon in response to arginine infusion [47]. This paradox appears to have been explained recently by the 'intra-islet hypothesis', which postulates that glucagon release in response to hypoglycaemia also requires a fall in intra-islet insulin concentration, and hence a reduction in tonic $\alpha$-cell inhibition [49]. In type 1 diabetes, intra-islet insulin concentrations are close to zero at all times and no such fall is possible. Support for this hypothesis is given by studies in non-diabetic humans: when islet insulin secretion is prevented from falling by administration of sulphonylurea [50], or suppressed in advance of hypoglycaemia by administration of diazoxide or somatostatin [51,52], the glucagon response to hypoglycaemia is diminished.

The relative importance of these endocrine responses in the prevention of severe hypoglycaemia has been reviewed by Cryer, based on studies in healthy adults in whom secretion of glucagon and growth hormone was inhibited by infusion of somatostatin and adrenergic effects were inhibited by combined α- and β-blockade, and in adults with bilateral adrenalectomy [53]. Hypoglycaemia was induced by bolus insulin injection, and the time taken for the blood glucose level to recover was determined for various combinations of hormonal suppression and replacement. These studies show persuasively that, in the absence of food ingestion, (i) sole adrenaline deficiency or blockade has little effect on recovery from hypoglycaemia; (ii) sole glucagon deficiency slows but does not abolish recovery from hypoglycaemia; (iii) combined glucagon and adrenaline deficiency/blockade abolishes recovery from hypoglycaemia. As described earlier, it seems that the glucagon response must inevitably be lost in type 1 diabetes. Recovery from hypoglycaemia therefore depends on (i) the adrenaline response, which diminishes with time; and (ii) symptom detection.

The symptoms of hypoglycaemia are diminished, and occur at lower blood glucose levels, both in diabetes of long duration by comparison with shorter duration [3,44,54] and in strict glycaemic control by comparison with poor glycaemic control [55,56]. Strict glycaemic control is also associated with attenuated catecholamine responses to hypoglycaemia [57]. An obvious possible mechanism is adaptation to hypoglycaemia, as patients who have strict glycaemic control and/or long duration of diabetes will have had greater cumulative exposure to hypoglycaemia. Confirmation of this is given by studies of the effects of antecedent hypoglycaemia on responses to hypoglycaemia in non-diabetic [58] and type 1 diabetic humans [59,60]. In each of these studies, a preceding period of hypoglycaemia resulted in attenuated symptoms and hormonal counterregulation during a hypoglycaemic clamp. In people with type 1 diabetes, careful avoidance of hypoglycaemia through meticulous insulin control has been shown to partially reverse this adaptation with respect to symptoms and adrenaline release; glucagon secretion remained negligible, as would be predicted by the intra-islet hypothesis [61,62,63].

In summary, impaired hypoglycaemia awareness is a common complication of insulin-treated diabetes, and appears to be a maladaptive response to recurrent episodes of

iatrogenic hypoglycaemia. The principle defences against hypoglycaemia are secretion of glucagon and adrenaline, and ingestion of food prompted by symptom detection. The first defence (glucagon) is abolished in type 1 diabetes, and the second (adrenaline) and third (symptoms) become impaired, but this would be of little consequence were it not for the cognitive effects of hypoglycaemia. If hypoglycaemia had no effect on cognitive function, then a diabetic person with impaired hypoglycaemia awareness would simply treat themselves when they detected symptoms at a lower blood glucose level. However, cognitive impairment does occur, which both prevents self-treatment and can cause dangerous or inappropriate behaviour. Therefore, continuing the argument of section 1.1, it can be argued that it is not hypoglycaemia *per se* but its effects on cognitive function that prevent people with diabetes simply increasing insulin doses until hyperglycaemia is abolished. Hypoglycaemic cognitive function is clearly a critical issue, and will be discussed in the next chapter.

## 1.7 Hyperglycaemia

Hyperglycaemia is the hallmark of diabetes, but its symptoms and effects on cognition have received less research attention than have the effects of hypoglycaemia. This is doubtless because hypoglycaemia is an acute state that can cause dramatic symptoms and endanger physical safety, whereas the symptoms and consequences of hyperglycaemia are more insidious or chronic. Nonetheless, there is potential value in researching acute hyperglycaemia:

i. Acute hyperglycaemia may have acute effects on mental or physical performance that have not been recognised or well described.

ii. Awareness of hyperglycaemic symptoms may allow individuals to take action to correct hyperglycaemia, diminishing any acute effects, and helping to improve chronic glycaemic control.

### 1.7.1 Effects of Hyperglycaemia on Cognition and Mood

A number of studies have examined cognitive function during acute hyperglycaemia. Cox *et al* assessed this in the field, by issuing patients with type 1 diabetes with hand-held computers containing electronic versions of cognitive function tests [64]. Patients were instructed to perform these tests immediately before routine blood glucose testing; cognitive performance was then analysed in five categories of blood glucose. Their results appear to show that a blood glucose >15 mmol/L is associated with impaired verbal fluency, arithmetical ability and simple reaction time. However, the statistical test (an ANOVA comparing all five categories) was inappropriate for this purpose.

In glycaemic clamp studies, Pais *et al* found no effect of mild hyperglycaemia (10.5 mmol/L) on memory and attention tasks in subjects with type 2 diabetes [65], and Gschwend *et al* found no effect of marked hyperglycaemia (20 mmol/L) on reaction time or Trail Making B performance in children with type 1 diabetes aged 9-19 years [66]. However, in a comprehensive study from Edinburgh, Sommerfield *et al* found that performance on Digit Symbol Substitution, Trail Making B, choice reaction time, and some aspects of working memory and attention were significantly impaired at 16.5 mmol/L in subjects with type 2 diabetes [67]. Furthermore, Sommerfield *et al* also

found an effect of hyperglycaemia on mood: in specific questionnaires, subjects indicated reduced levels of happiness and alertness, and more agitation.

### 1.7.2 Symptoms of Hyperglycaemia

Clinical experience tells us that the symptoms of hyperglycaemia include thirst, polyuria and dryness of mucous membranes [68]. Although the value of clinical experience and anecdotal evidence should not be underestimated, only a few research studies have attempted to corroborate this.

Pennebaker *et al* and Cox *et al*, in studies from the same centre, asked patients with type 1 diabetes to rate the intensity of certain symptoms before blood glucose testing, both in hospital and in the community [69,70]. Symptom intensity could then be paired with blood glucose result, to identify symptoms of hypo- and hyperglycaemia. The only symptom that was clearly associated with hyperglycaemia was "Dry eyes, nose or mouth", though "Sweet taste" and "Salivation" also had some endorsement. The studies were rather odd, in that other obvious symptoms of hyperglycaemia such as thirst and polyuria were not included, and hyperglycaemia appeared to be defined as a blood glucose level >110mg/dL (6.1 mmol/L). The same group have developed an educational programme, "Blood Glucose Awareness Training" (BGAT), based in part on teaching people with diabetes to recognise the symptoms of hypo- and hyperglycaemia. One randomized, controlled trial showed no benefit of BGAT for either hypoglycaemia frequency or $HbA_{1c}$ [71]. A later trial showed a reduction in frequency of severe hypoglycaemia, and improved detection of both hypoglycaemia and hyperglycaemia, though again without benefit to $HbA_{1c}$ [72].

Overall, the symptoms of hyperglycaemia have not received detailed assessment. Unlike hypoglycaemic symptoms, the usual sensations that are experienced by people with diabetes have not been clearly described, and their physiological causes are not understood beyond an assumption that osmotic fluid shift is important. Although the main focus of this thesis is cognition during hypoglycaemia, Chapter 7 describes a study of hyperglycaemic symptoms in people with diabetes.

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# Chapter 2

# Cognitive Function and Hypoglycaemia

## 2.1 Brain metabolism

The human brain normally depends entirely on glucose for its energy. Confirmation of this is given by the respiratory quotient (RQ), the ratio of $CO_2$ produced to $O_2$ consumed. This approaches 1.0 when carbohydrate oxidation is the sole fuel, and for the brain the RQ has been experimentally calculated as 0.97 [1], with a small proportion of glucose ending up in other chemicals including the neurotransmitters GABA, acetylcholine, aspartate and glutamate [2]. The glycogen content of the brain is sufficient for only 4-5 minutes of normal energy use [3]. Potential fuels other than glucose include ketone bodies, lactate and amino acids, although the contribution that these make to brain metabolism in the acute phase is thought to be minimal [3]. Acute hypoglycaemia therefore causes immediate energy deficiency in the brain, rescued only by correction of the blood glucose level. This energy deficiency is assumed to be the cause of cerebral dysfunction, of which one manifestation is cognitive impairment.

The blood-brain barrier prevents simple diffusion of water-soluble molecules such as glucose, which cross instead by facilitated diffusion via the glucose transporters GLUT1 to GLUT5. Transport of glucose has been considered insulin-independent on the basis of studies showing no increase with hyperinsulinaemia [4,5,6] although recent evidence has indicated that physiological insulin concentrations increase brain glucose uptake by comparison with sub-physiological concentrations [7].

## 2.2 Cognitive impairment during hypoglycaemia

### 2.2.1 Confirmation of cognitive dysfunction during hypoglycaemia

Fletcher & Campbell provided perhaps the earliest report of cognitive dysfunction [8], describing instances of accidental hypoglycaemia associated with the newly-discovered insulin therapy. The authors described loss of fine motor function, dysarthria and confusion, and also noted that the blood glucose level at which these changes occurred varied from patient to patient. Numerous descriptions of hypoglycaemic cognitive dysfunction followed, although the first known attempt to study this formally was not until 1975 [9]. This study reported impaired motor coordination and word recall, and slower (but not less accurate) performance on a reasoning task. Holmes and colleagues expanded on this work in the 1980s, studying both diabetic and non-diabetic subjects, and finding that relatively mild hypoglycaemia (3.0 to 3.4 mmol/l) resulted in impairment of several different aspects of cognitive function, including reaction time, mathematical computation, and verbal fluency [10,11,12,13].

The literature in this area expanded rapidly over the next decade, with many aspects of cognitive function being studied during hypoglycaemia. Results of studies that have examined cognitive function during moderate hypoglycaemia are summarized in Table 2.1 (excluding studies in which glycaemic thresholds were estimated, discussed in section 2.3). This subject has been comprehensively reviewed by Deary [14,15], but broadly speaking, the following cognitive functions have consistently been shown to be impaired by moderate hypoglycaemia (2.0 to 3.4 mmol/l): visual and auditory reaction time, mathematical computation, verbal fluency, and number recall. Other aspects of cerebral function, such as fine motor control, have also shown impairment, though there is some debate as to whether these meet the generally understood meaning of 'cognitive function'.

| Cognitive test | Blood glucose level (mmol/l) | | | |
|---|---|---|---|---|
| | Non-diabetic | | Diabetic | |
| | Impaired | Unimpaired | Impaired | Unimpaired |
| **Finger tapping** | 3.05 [16] | 2.0 [17]<br>3.1 [11]<br>3.4 [18] | 2.0 [17] | 2.0 [19]<br>2.7 [20]<br>3.1 [13] |
| **Verbal fluency** | | 3.1 [11] | 2.0 [19] | |
| **Digit recall** | | 2.2 [21]<br>3.3 [10] | 2.0 [19] | 2.2 [21] |
| **Word recall** | | 2.2 [21] | | 2.2 [21] |
| **Simple visual reaction time** | 2.0 [17]<br>2.5 [22]<br>2.8 [23]<br>3.3 [10] | 3.1 [12]<br>3.4 [18] | 2.0 [17]<br>2.5 [22]<br>2.7 [20]<br>2.8 [23] | |
| **Simple auditory reaction time** | | 3.4 [18] | 3.1 [13] | |
| **Choice visual reaction time** | 2.5 [24]<br>2.5 [25]<br>2.65 [26] | 3.4 [18] | | |
| **Choice auditory reaction time** | | | 3.1 [13] | |
| **Digit symbol substitution** | 2.5 [24]<br>2.5 [25]<br>2.5 [27,28]<br>2.5 [28]<br>2.6 [29]<br>3.4 [18] | 2.0 [17]<br>2.5 [25] | 2.0 [17]<br>2.5 [30]<br>2.6 [31]<br>2.6 [32]<br>2.8 [33] | |
| **Trail making B** | 2.5 [24]<br>2.5 [27]<br>2.5 [28]<br>3.4 [18] | 2.65 [26]<br>2.5 [25] | 2.0 [19]<br>2.2 [34]<br>2.5 [35]<br>2.5 [30]<br>2.6 [36]<br>2.6 [31]<br>2.6 [32]<br>2.6 [29]<br>2.8 [20] | |
| **Stroop ink colour tasks** | 2.65 [26]<br>3.05 [16]<br>3.1 [11]<br>3.1 [13] | | | |

**Table 2.1.** Summary of studies reporting performance on cognitive function tests as impaired and unimpaired at various blood glucose levels. 'Impaired' generally means a statistically significant ($P<0.05$) reduction in score; 'unimpaired' generally means failure to achieve the same.

### 2.2.2 The meaning of cognitive function testing

When a hypoglycaemia study is devised, the choice of cognitive function test is limited by a number of practical requirements. It must be possible to perform the test in a recumbent position with one hand; the test must yield an objective, numerical score; and usually, the test duration must be no more than a few minutes. The duration of the test is particularly important in studies that seek to determine glycaemic thresholds for cognitive dysfunction, as testing at multiple time points is required. As Table 2.1 shows, four of the most commonly-used tests are choice reaction time, Trail-Making B, Digit Symbol Substitution and the Stroop ink colour tasks. There are at least two problems when it comes to interpreting performance on these tasks.

Firstly, for these and most other tests, there is no agreed account of the cognitive abilities which are engaged. For example, in Trail-Making B subjects are required to connect letters and numbers in the sequence 1-A-2-B-3-C-etc. from a 5x5 grid in which they have been randomly distributed. Consideration of the task suggests that working memory (remembering which letter or number comes next), visual scanning (finding that letter or number) and hand-eye coordination (joining letters and numbers in a 'trail') must all be involved. These abilities seem to be separate, but this is little more than intuition, and there is no guarantee that they utilize different cerebral pathways. Furthermore, if Trail-Making B performance is impaired during hypoglycaemia, we cannot say whether this is because of effects on all, some, or only one of these cognitive abilities. It is possible that performance decrements are due to a very non-specific effect such as global cognitive deterioration, or even difficulty paying attention; moderate hypoglycaemia has been demonstrated to impair performance on tests considered to measure attention in both diabetic [37] and non-diabetic subjects [38].

Secondly, for most of the commonly-used tests, what is measured is cognitive speed. Reaction time, the Stroop tasks and Trail Making are measured as completion time. Digit Symbol Substitution and finger-tapping are measured as the number of substitutions or taps completed in a set time. Thus, most studies that have reported cognitive impairment during hypoglycaemia have actually shown *slowing* of cognitive function. Omitting this detail ignores the potential trade-off between speed and accuracy or absolute ability, and may overstate the real-life importance of the

hypoglycaemic effect. Holmes *et al* reported that subjects completed fewer mathematical computations and Stroop tasks during hypoglycaemia, but achieved the same proportion of correct answers [10,11]. In studies of attention during hypoglycaemia, completion time rather than accuracy was impaired [37,38], and studies using heterogeneous test batteries have reported greater deterioration on timed than non-timed tests [21,34].

Two strategies have been adopted to attempt to sidestep the uncertain meaning of most cognitive tests. The first involves narrowing the experimental scope to measurement of fundamental brain processes during hypoglycaemia. Many researchers have studied the effects of hypoglycaemia on the P300 wave, which is a brain evoked potential that is detected approximately 300 milliseconds after presentation of an auditory or visual stimulus. Delay or attenuation of the P300 wave is associated with poorer cognitive performance, and has been reported during hypoglycaemia in numerous studies [39,40,41,42,43,44,45]. Aspects of auditory information processing, including volume and temporal discrimination but not pitch discrimination, are impaired in both diabetic and non-diabetic subjects [32,46]. Similarly, aspects of visual information processing, including inspection time, visual change detection and movement detection but not visual acuity, are impaired in diabetic and non-diabetic subjects [29,31,47]. However, the difficulty here is in the real-life relevance of these results. Activities in daily life result are complex and are achieved by the integration of multiple cerebral processes. There is no evidence that a statistically significant increase in visual inspection time under laboratory conditions may be translated into (for example) a meaningful deterioration in driving ability, and thus the ecological validity of these cognitive tests is questionable [48].

The second strategy is to broaden the cognitive function tests, to the point that a minimum of extrapolation to daily activities is required. Mathematical computation is, as mentioned above, impaired during hypoglycaemia [14,15]. Another ability that is important in daily life, and has been studied during hypoglycaemia, is driving. Cox *et al* performed two studies in subjects with type 1 diabetes using a driving simulator, which is not a perfect replica but is a much closer approximation than measurements of reaction time and visual inspection speed [49,50]. Overall driving performance was

impaired by mild hypoglycaemia (3.6 mmol/l in the earlier study and ~3.0 mmol/l in the latter), with a tendency to veer off course, drive too fast and brake inappropriately. There is some evidence that motor vehicle accident rates are higher for diabetic drivers than for non-diabetic drivers [51], and although the literature is not consistent on this subject [52,53], this does support a real-life correlation for laboratory hypoglycaemia studies.

### 2.2.3 Experimental design and methodology

A major problem with the body of research on hypoglycaemic cognitive function is the difficulty of comparing results from different studies. There are many instances where different studies appear to show conflicting results – for example, performance on a particular cognitive test being impaired during hypoglycaemia in one study but not in another. Much of the disagreement is likely to have resulted from deficiencies and differences in experimental methods, as discussed by Heller & Macdonald [54], which include:

*Degree of hypoglycaemia.* The target blood glucose has varied widely, between 1.5 and 3.6 mmol/l.

*Blood sampling.* Arterial blood glucose measurement is the direct measure of glucose supply to tissues, but arterial cannulation is a relatively difficult and painful procedure. Venous blood glucose is much easier to measure, but is not perfectly correlated with arterial glucose, as the proportion of glucose taken into tissues increases with hypoglycaemia. Many studies have used arterialized venous blood sampling, where venous blood is taken from a hand that is placed within a heated box to increase blood flow and create a partial arteriovenous shunt [55]. This reduces, but does not negate, differences between arterial and venous sampling.

*Measurement of glucose concentration.* Glucose concentration may be measured in whole blood or in plasma. Plasma glucose concentrations are typically 10-20% higher than the equivalent whole blood concentrations [56].

*Method of induction of hypoglycaemia.* In earlier studies, uncontrolled hypoglycaemia was induced by bolus injection of insulin. The rate of fall of blood glucose could not be kept constant to ensure equivalent hypoglycaemia in all subjects, nor could the effects of

prolonged hypoglycaemia be studied. Later, researchers infused insulin at a variable rate aiming to maintain a target blood glucose level, but the delayed effect of insulin infusion adjustments meant that this remained inaccurate. The hyperinsulinaemic glucose clamp has been in general use for the last 15 years [57], and involves infusion of insulin at a high, fixed rate which is intended to saturate insulin receptors and deliver a maximal hypoglycaemic response. Simultaneously, glucose is infused at a variable rate to achieve target blood glucose concentrations. This method allows the design of studies such that subjects are exposed to identical hypoglycaemia and insulinaemia, and although more complicated, is clearly preferable.

*Euglycaemia control studies.* Clamp studies are arduous for subjects, and over their course subjects may suffer from fatigue or boredom. Conversely, if cognitive function tests are repeated, then a practice effect may occur. For these reasons, it is not adequate to compare performance during hypoglycaemia with baseline performance. Instead, the effects of hypoglycaemia should be determined by comparing performance at equivalent time points in hypo- and euglycaemic studies. Earlier studies did not usually include euglycaemic control studies, and their conclusions are in consequence less robust.

*Subject selection.* Studies of healthy (non-diabetic subjects) may be confounded by variables such as age, sex and race. Studies of diabetic subjects may also be confounded by duration of diabetes and diabetic complications. Perhaps more importantly, as will be discussed later, exposure to hypoglycaemia itself modifies subsequent responses to hypoglycaemia, and it is extremely difficult or impossible to ensure that two different groups of subjects have equivalent prior hypoglycaemia exposure.

*Statistical power.* Lack of statistically significant cognitive dysfunction is not evidence that hypoglycaemia has no effect on a cognitive process, unless appropriate power calculations are also reported. Studies in this research area have rarely or never reported power calculations.

### 2.2.4 Sensitivity of different cognitive processes to hypoglycaemia

It has been suggested that performance on complex tasks involving higher cerebral processes is impaired to a greater extent by hypoglycaemia than performance on simple perceptual and motor tasks [14]. For example, finger tapping has frequently been unaffected during hypoglycaemia (Table 2.1). Holmes *et al* reported greater

deterioration in choice reaction time (which requires a decision) than in simple reaction time during hypoglycaemia [12]. Stevens *et al* reported impairment of more complex tasks such as Digit Symbol Substitution and Trail Making during hypoglycaemia, while simple reaction time and finger tapping were unaffected [18].

Those interpretations are not wholly persuasive: describing finger tapping as a cognitive function test is at least questionable, and Table 2.1 demonstrates that finger tapping and simple reaction time have been slowed in some studies during hypoglycaemia. For the reasons outlined above, comparing results from different studies is fraught with pitfalls due to differing methodology and subjects. Even when two cognitive tests are used within the same study, there are difficulties in comparing them. If the performance decrement for one test achieves statistical significance and the other does not, that does not mean that there is a statistically significant difference between them, although this interpretation has commonly been presented. As yet, there is no statistically robust evidence that hypoglycaemia has a greater effect on performance on any one test than any other.

### 2.2.5 Conclusions regarding cognitive function during hypoglycaemia

Research on the effects of moderate hypoglycaemia on cognitive function has been limited by lack of experimental consistency, small studies, inadequate statistical analysis and uncertainties about what is being measured by cognitive tests.

The following conclusions relating to hypoglycaemia at about 2.0 to 3.0 mmol/l are supported:

- Hypoglycaemia results in impairment or slowing of fundamental sensory processes, including visual and auditory change detection and critical stimulus threshold.
- Hypoglycaemia impairs mathematical computation, verbal fluency and number recall.
- Hypoglycaemia results in slowing of performance on many tasks that appear to test cognitive function, including visual and auditory reaction time, Trail Making B, Digit Symbol Substitution and Stroop ink colour tasks.

There is some evidence for the following interpretations:

- Hypoglycaemia impairs driving performance (experimental evidence is restricted to driving simulators).
- Hypoglycaemia has more effect on complex tasks than on simple ones (the evidence for this lacks statistical corroboration).
- Hypoglycaemia slows or impairs all cognitive functions (no test that has been used in more than one study has been consistently unaffected by hypoglycaemia).

## 2.3 Glycaemic thresholds for cognitive dysfunction

### 2.3.1 Methodology for estimation of glycaemic thresholds

In the studies already discussed, researchers examined whether cognitive performance was impaired at a particular level of blood glucose. This approach does not tell us at what level of blood glucose cognitive dysfunction begins. A different approach is to use a stepped hypoglycaemic clamp, whereby the blood glucose concentration is lowered in a series of steps, with cognitive function assessed at each glycaemic 'plateau' (Figure 2.1). In this way, the glycaemic threshold for cognitive dysfunction may be estimated. There are semantic difficulties with the term 'threshold': in its metaphorical origin, the threshold must be stepped over, and so the higher the threshold the harder it is to cross, and the more extreme the situation represented. A 'higher glycaemic threshold' could, quite logically, be taken to mean a lower blood glucose level. Terms such as 'higher threshold' and 'lower threshold' will be avoided hereafter, though 'threshold' has been kept as a useful shorthand for 'the level of blood glucose at which [an event] occurs'.

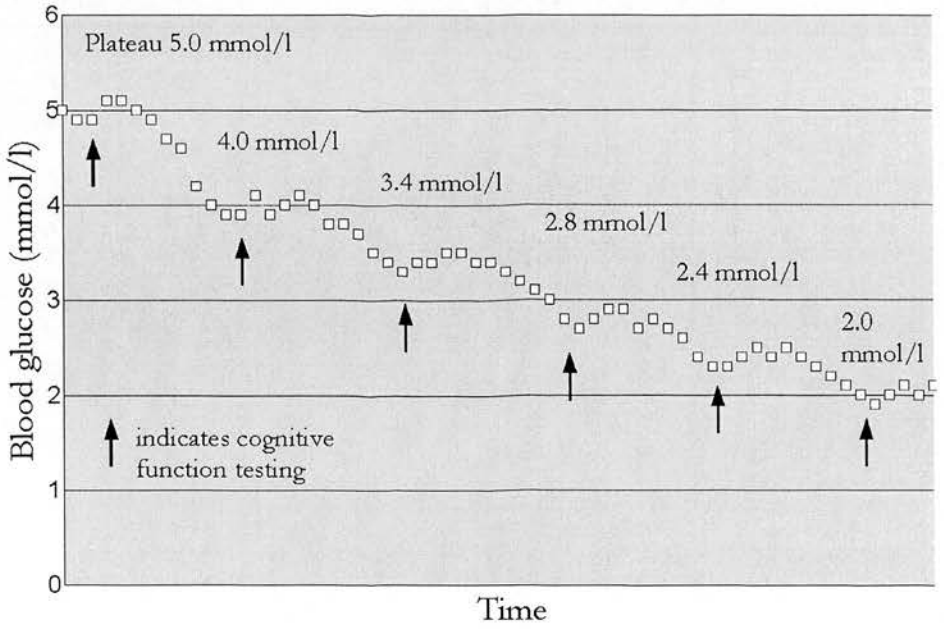


**Figure 2.1.** Illustration of the stepped glycaemic clamp method for estimation of thresholds for cognitive dysfunction.

Glycaemic thresholds for cognitive dysfunction were first calculated by Pramming *et al*, although their method of analysis was unusual and has not been repeated [19]. The technique adopted by Schwartz *et al* has persisted [58]. First, the standard deviation of the baseline (euglycaemic) scores on cognitive tests for the entire subject group is determined, and significant deterioration is thereafter defined as a decline greater than two of these standard deviations. Second, for each individual subject, the point at which their cognitive score falls below their baseline score by more than this pre-specified amount is identified. Third, the mean actual blood glucose during that plateau of the clamp for that individual is calculated. This blood glucose level (rather than the target) is taken to be the glycaemic threshold for the individual.

There are flaws with this method, which have not been widely acknowledged. Firstly, the estimated blood glucose for the onset of cognitive dysfunction must be lower than the true blood glucose level. For example, if cognitive dysfunction is not detected at 2.8 mmol/l but is detected at 2.4 mmol/l, the threshold must lie somewhere between these values. Secondly, because the clamp technique does not give perfect control of blood glucose, achieved levels are used rather than the target levels. Again, this may be illustrated by an example, in which the true threshold for cognitive dysfunction is 2.6 mmol/l, and clamp targets include 2.4 and 2.8 mmol/l. The 2.4 mmol/l plateau will be the first to show cognitive dysfunction, but different subjects' thresholds may be variously recorded as 2.3, 2.4, 2.5 mmol/l, depending on the achieved blood glucose level. This produces apparent individual differences, which are actually experimental artefacts. Additionally, it creates the illusion that the threshold estimate is a continuous variable whereas it is really categorical, which has implications for statistical methods.

### 2.3.2 Glycaemic thresholds in non-diabetic subjects

Table 2.2 summarizes threshold estimates for cognitive dysfunction. In non-diabetic subjects these have ranged between 2.3 and 3.1 mmol/l. Estimates in diabetic subjects are similar, although for reasons that will be discussed later, these are very dependent on the patient group studied. There are no obvious outliers (i.e. cognitive function tests that show impairment at a higher or lower blood glucose than the other tests).

| Cognitive test | Glycaemic threshold (mmol/l) | |
|---|---|---|
| | Non-diabetic | Diabetic |
| **Finger tapping** | 2.4 [59] | |
| **Simple visual reaction time** | | 2.2 [34]<br>2.8 [33] |
| **Choice visual reaction time** | 2.8 [59]<br>2.9 [60]<br>3.1 [61] | 2.9 [60]<br>3.0 [62]<br>3.2 [61] |
| **Trail-making B** | 2.3 [21] | Good control<br>2.3 [21]<br>Poor control<br>2.7 [21] |
| **Stroop ink colour tasks** | 2.5 [59] | |
| **Summary cognitive function score** | 2.5 [63]<br>2.6 [64]<br>2.65 [77]<br>2.7 [65]<br>2.7 [66]<br>2.8 [67]<br>3.0 [68] | Hypo aware<br>2.69 [69]<br>Hypo unaware<br>2.39 [69] |

**Table 2.2**. Summary of studies estimating glycaemic thresholds for cognitive dysfunction.

Despite its limitations, estimation of glycaemic thresholds has provided useful information. Mitrakou *et al* calculated thresholds in non-diabetic subjects for counterregulatory hormone release (3.6-3.8 mmol/l), autonomic symptoms (3.2 mmol/l), neuroglycopenic symptoms (2.8 mmol/l) and cognitive dysfunction (2.7 mmol/l) [66]. Importantly, these threshold estimates were compared and found to differ with high significance (P=0.0001 for hormones vs autonomic symptoms; P=0.018 for autonomic vs neuroglycopenic symptoms; P=0.004 for autonomic symptoms vs cognitive dysfunction). The methodological deficiencies mentioned above will tend to reduce the significance of any comparisons; statistically significant comparisons are therefore relatively trustworthy. Mitrakou *et al*'s study is important because it is the only concrete evidence that in non-diabetic subjects, cognitive dysfunction occurs at a lower blood glucose level than endocrine counterregulation and symptom generation. Similar threshold estimates showing the same hierarchy were obtained by Fanelli *et al* and Mokan *et al,* although there were no statistical comparisons between thresholds to

confirm that cognitive dysfunction occurs at a lower blood glucose level than symptoms or hormonal counterregulation [63,69]. These results are illustrated in Figure 2.2.

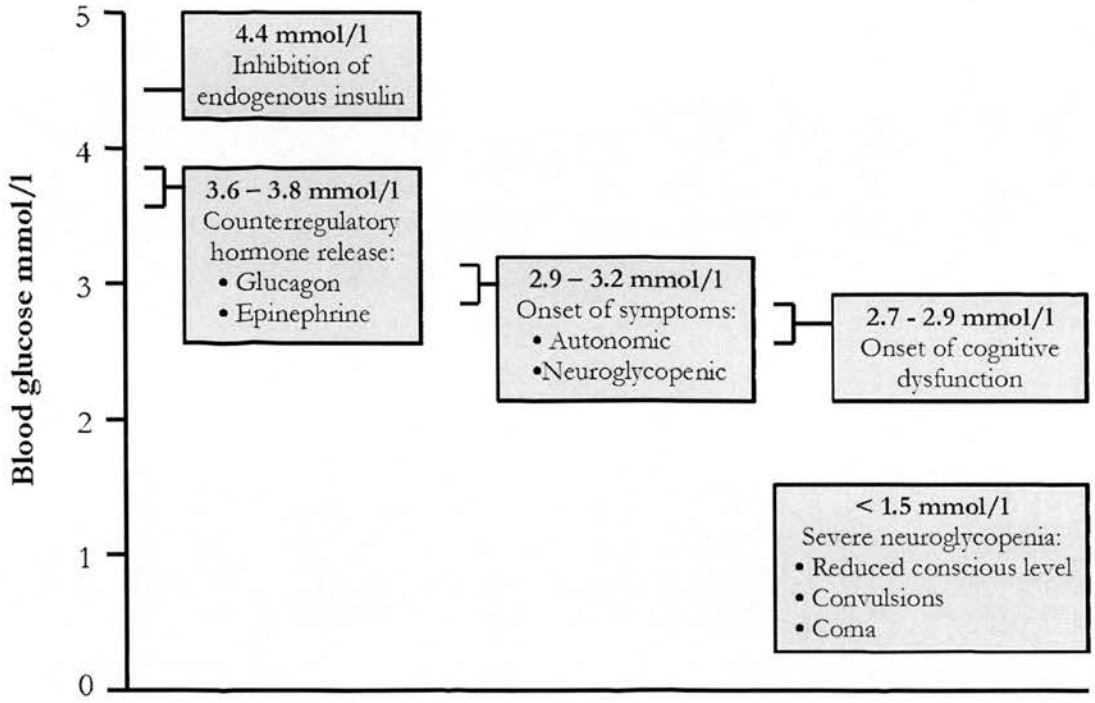


**Figure 2.2.** Thresholds of responses to hypoglycaemia in non-diabetic subjects. Based on [63,66,69].

### 2.3.3 Conclusions regarding glycaemic thresholds in non-diabetic subjects

Although methods of estimation of glycaemic thresholds have some inherent flaws, all estimates for the onset of cognitive dysfunction in non-diabetic subjects are between 2.3 and 3.1 mmol/l, with most being around 2.7-2.9 mmol/l. Although it seems intuitively likely that complex tasks would be more susceptible to hypoglycaemia, with reference to (for example) the effects of traumatic brain damage or alcohol intoxication, there is no evidence that thresholds vary for different cognitive processes.

Importantly, there is a hierarchy of responses to progressive hypoglycaemia in non-diabetic subjects. As blood glucose falls, endocrine counterregulation is the first

response, followed by symptom generation. Cognitive dysfunction occurs at a still lower blood glucose level in non-diabetic subjects.

## 2.4 Speed of onset of, and recovery from, hypoglycaemic cognitive dysfunction

Evans *et al* induced hypoglycaemia in non-diabetic subjects, and measured cognitive function, symptoms and counterregulatory responses immediately and after 20 minutes of sustained hypoglycaemia [26]. There was statistically significant impairment of cognitive function immediately, whereas counterregulation and symptoms only achieved significance at 20 minutes. If the possibility of type 2 statistical error is ignored, it can be inferred that even people with normal hypoglycaemia awareness may develop neuroglycopenia before symptoms if blood glucose falls rapidly.

Cognitive function does not recover immediately on correction of hypoglycaemia. Several studies have reported delays of 45 to 90 minutes before recovery of reaction time or P300 amplitude, but each had a flaw such as failure to determine statistical significance or absence of a euglycaemic control arm [17,22,23,24,70]. Fanelli *et al* reported data apparently showing residual impairment 60 minutes after hypoglycaemia, although their statistical analyses ignored the euglycaemic control arm and simply compared scores with baseline [71]. Evans *et al* reported a methodologically and statistically robust residual impairment 20 minutes after restoration of euglycaemia [26]. More recently, Nicola Zammitt and I have shown that a delayed effect of hypoglycaemia on choice reaction time persists as long as 75 minutes after correction of hypoglycaemia [72].

## 2.5 Cognitive adaptation to hypoglycaemia

### 2.5.1 Acute adaptation of cognitive function during hypoglycaemia

Several studies have reported acute adaptation of cognitive function to hypoglycaemia, but statistical confirmation was often lacking. For example, Kerr *et al* measured reaction time repeatedly at an arterialized venous blood glucose of 2.8 mmol/l in type 1 diabetic subjects, and reported that reaction time was significantly prolonged after 40 minutes of hypoglycaemia, but not at 20, 65 or 90 minutes [73]. The loss of statistical significance was taken as evidence of cerebral adaptation, although inspection of their data suggests that reaction time was prolonged during hypoglycaemia at all times, with only the comparison at 40 min reaching the critical $P<0.05$. The study may simply have been underpowered to reliably detect hypoglycaemic cognitive decline.

More convincingly, Kerr *et al* lowered blood glucose to 3.5 for an hour and then to 3.0 mmol/l for a further hour in non-diabetic subjects, and their data are approximated in Figure 2.3 [74]. The baseline (278 ms) and peak (349 ms) reaction times were significantly different ($P<0.01$). Comparisons of the peak and final (287 ms) reaction times were not reported; comparison of the published data by the standard error method gives $P=0.004$. A learning effect was not excluded by comparing the euglycaemia reaction times (unpublished) at each time point. Even so, the data are strongly suggestive of acute adaptation, in this case after 150 minutes of mild hypoglycaemia.

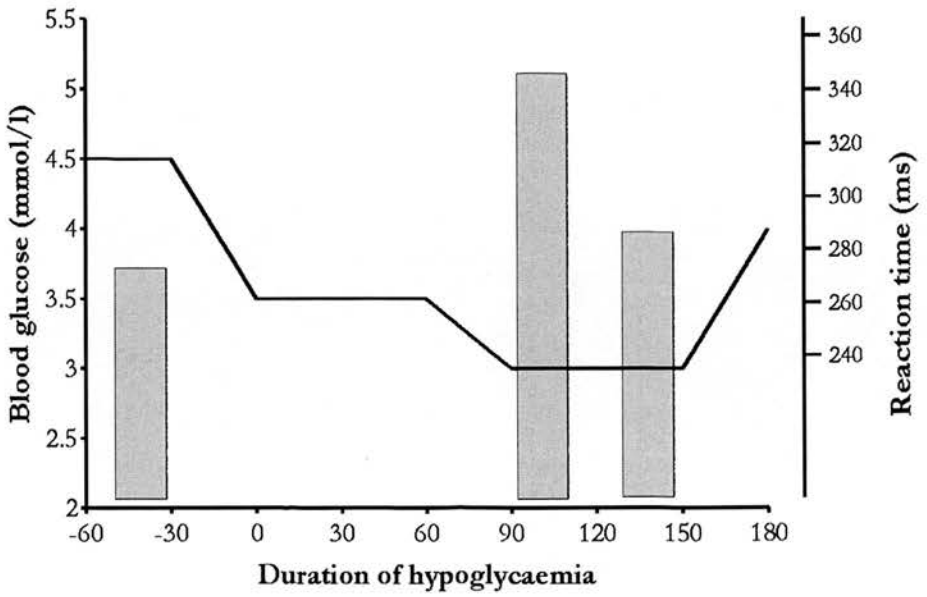


**Figure 2.3.** Reaction time during stepped hypoglycaemia, approximated from [74].

By contrast, Gold *et al* induced hypoglycaemia (arterialized venous blood glucose 2.5 mmol/l) in 24 non-diabetic subjects, and determined cognitive decrement with reference to euglycaemic control studies. The cognitive decrement did not differ significantly after 0 and 40 minutes of hypoglycaemia, suggesting that no adaptation occurred during this period [24]. Evans *et al* found no change in cognitive function between 0 and 60 minutes of hypoglycaemia (arterialized venous plasma glucose 2.65 mmol/l) in non-diabetic subjects, although with only eight subjects the study lacked statistical power [26].

An impressive study by Boyle *et al* supports the concept of acute adaptation to hypoglycaemia [16]. Brain glucose uptake was determined by measuring glucose concentrations in arterial and jugular venous blood, and multiplying the difference by the cerebral blood flow, estimated by a tracer method. In non-diabetic subjects, brain glucose uptake and cognitive function were measured during a stepped hypoglycaemic clamp on day 1, and again on day 4 after 56 hours of continuous hypoglycaemia at approximately 3.0 mmol/l. The clamp plateaux were at 4.7, 4.2, 3.6, 3.0 and 2.5 mmol/l (arterial plasma), and cognitive tests were immediate and delayed recall of a word list,

finger tapping and Stroop task performance. Brain glucose uptake was significantly reduced at a blood glucose level of 3.6 mmol/l and below on day 1, but there was no significant fall in brain glucose uptake at any blood glucose level on day 4. Stroop task performance was significantly impaired at 3.0 mmol/l on day 1, but not until 2.5 mmol/l on day 4, and finger tapping was impaired at 3.0 mmol/l on day 1 but not at any blood glucose level on day 4. The significance levels for day 1 vs day 4 cognitive performance were reported as P=0.04 (Stroop) and P=0.004 (finger tapping), although it is not clear if these comparisons took baseline performance on each day into account to exclude a learning effect. However, the increased brain glucose uptake seems robust, and the study strongly suggests that cognitive adaptation to hypoglycaemia occurs through this mechanism.

### 2.5.2 Adaptation of cognitive function following recent hypoglycaemia

Several studies have reported that hypoglycaemia reduces cognitive impairment during subsequent hypoglycaemia. The typical design for these studies is as follows: (i) the subject is first rendered hypoglycaemic for 1-2 hours, and after a set interval of hours or days, hypoglycaemia is induced again and cognitive dysfunction measured; (ii) after a fortnight or more the sequence is repeated, but with a euglycaemic first clamp; (iii) hypoglycaemic cognitive dysfunction is compared for studies following first clamp hypoglycaemia and first clamp euglycaemia.

Table 2.3 summarizes some of these studies. It is worth remembering that when two or more tests are employed, the P-value for significance for any one test should be subject to a Bonferroni correction, i.e. divided by the number of tests employed. Thus, Mellman *et al*'s P<0.03 for attenuated cognitive impairment on the Logical Memory task is not strictly significant, as two tests were employed and the critical value should be P=0.05/2=0.025; on the other hand, with only nine subjects, failure to find a significant difference for Digit Symbol Substitution may have been due to lack of statistical power [75]. Fanelli *et al* avoided the problem of multiple comparisons by generating a summary score for all cognitive function tasks, similar to the primary endpoint of clinical trials, which was significantly preserved seven hours after antecedent

hypoglycaemia [76]. Fruehwald-Schultes *et al* found significant preservation of all their cognitive measures 24 hours after antecedent hypoglycaemia [45]. Only George *et al* did not report any preservation of cognitive dysfunction, but this was a small study, and it is possible that the duration of adaptation after a single hypoglycaemic episode is less than two days [62]. Overall, it seems likely that acute adaptation of cognitive function follows within seven hours (and possibly within two hours) of hypoglycaemia.

| Study | Subjects in each group | Antecedent hypoglycaemia | Interval | Cognitive functions showing preservation | Cognitive functions not showing preservation |
|---|---|---|---|---|---|
| Mellman *et al* [75] | 9 non-diabetic | 2 hr at 3.2 mmol/l | Approx. 2 hours | Logical Memory (P<0.03) | Digit symbol substitution |
| Fanelli *et al* [76] | 15 T1DM | 3.5 hr at 2.7 mmol/l | Approx. 7 hours | Summary cognitive function (P=0.006)<br>Delayed non-match to sample (P=0.037)<br>Stroop arrow-word (P=0.039) | Paced serial addition (PASAT)<br>Paragraph recall |
| Fruehwald-Schultes *et al* [45] | 15 non-diabetic | 2.5 hr at 3.1 mmol/l | 24 hr | P300 amplitude (P<0.005)<br>Auditory reaction time (P<0.05)<br>Word recall (P<0.005) | |
| George *et al* [62] | 8 T1DM | 2 hr at 2.8 mmol/l | 2 days | | Choice visual reaction time |

**Table 2.3.** Studies reporting preservation of hypoglycaemic cognitive function after antecedent hypoglycaemia.

### 2.5.3 Adaptation of cognitive function to hypoglycaemia in people with insulin-treated diabetes

Clinical experience relates that people with tightly-controlled diabetes maintain cognitive function during quite profound hypoglycaemia. Experimental data has been patchy, but has tended to support this observation. Jones *et al* reported that in intensively-treated type 1 diabetic subjects, a much lower blood glucose level (2.2 mmol/l) was needed to achieve a statistically significant attenuation of the P300 evoked brain potential than in subjects with conventionally-treated type 1 diabetes (3.5 mmol/l) and without diabetes (3.0 mmol/l) [77]. However, there was no formal between-groups statistical test.

In the largest study of the type, Mokan *et al* studied 32 type 1 diabetic subjects with normal hypoglycaemia awareness, 11 with impaired awareness and 19 non-diabetic controls [69]. Cognitive function tests were Trail Making A&B, Stroop tasks, simple and choice reaction time, verbal memory and forward and backwards digit span. Despite this comprehensive battery, cognitive scores were summarized and reported as a single $z$-score; this avoids the problem of multiple statistical comparisons, but markedly reduces the available information, such that one has to question why so many cognitive tests were included in the first place. A stepped glycaemic clamp was utilized, as described in section 2.5.1. Blood glucose levels for the onset of reactions to hypoglycaemia were generally similar in controls and diabetic subjects with normal awareness. Compared with subjects with normal awareness, subjects with impaired awareness had lower blood glucose levels for the onset of autonomic symptoms (2.33 vs 3.61 mmol/l, $P<0.01$), neuroglycopenic symptoms (2.48 vs 2.83 mmol/l, $P<0.03$) and cognitive dysfunction (2.39 vs 2.69 mmol/l, $P<0.01$). Despite apparent cognitive adaptation to hypoglycaemia, the blood glucose interval between the onset of symptoms and the onset of cognitive dysfunction in aware subjects (3.61 vs 2.69 mmol/l) seems to have been lost in subjects with impaired hypoglycaemia awareness (2.48 vs 2.39 mmol/l). However, this was not subject to statistical corroboration.

Weinger *et al* conducted a similar but smaller study, with 8 subjects with relatively well-controlled diabetes, 9 with relatively poorly-controlled diabetes, and 10 controls [21]. No significant between-group differences in the blood glucose levels for cognitive dysfunction were observed, though significant differences were detected for autonomic symptoms and counterregulatory hormones. The latter study had substantially fewer subjects than in Mokan *et al*, which may explain the different results. Also, at least one of their tests was clearly too easy: the word recall test measured recall of just three words after five minutes.

If people with relatively tightly-controlled diabetes show less cognitive dysfunction during hypoglycaemia, a plausible explanation is cerebral adaptation secondary to frequent prior hypoglycaemia. A possible mechanism is increased blood-brain glucose transport. Studies in rats have reported upregulation of cerebral GLUT1 transporters after chronic hypoglycaemia (of several days' duration), although no changes were seen

with acute hypoglycaemia [78,79]. In a complementary study to that cited in section 2.5.1, Boyle *et al* measured brain glucose uptake at 3.0 mmol/l (arterial plasma glucose) in non-diabetic subjects and subjects with well-, averagely- and poorly-controlled diabetes. Brain glucose uptake was preserved in subjects with well-controlled diabetes, who by inference may have had frequent prior exposure to hypoglycaemia, but fell by around 20% in other diabetic and non-diabetic subjects [80]. Studies using positron-emitting glucose tracers to measure brain glucose uptake have not replicated these findings [81,82], and indeed, one recent study reported a *decrease* in cerebral glucose utilization during hypoglycaemia in hypoglycaemia-unaware subjects [83]. It is hard to reconcile these very different findings; there is evidence that brain glucose estimates by PET and arteriovenous difference techniques may diverge, but no gold standard against which they may be compared to determine which studies are more reliable [84].

### 2.5.4 Evidence for adaptation of cognitive function to hypoglycaemia from interventional studies

It would be unethical to intentionally cause multiple episodes of hypoglycaemia, in order to find out whether subjects develop resistance to its cognitive effects. However, the reverse approach of strictly avoiding hypoglycaemia is ethical, though labour intensive. In two separate studies, Fanelli *et al* studied subjects with type 1 diabetes and impaired awareness of hypoglycaemia, and also non-diabetic control subjects [65,67]. The magnitude of, and blood glucose levels for, the symptomatic, hormonal and cognitive effects of hypoglycaemia were determined using the stepped glycaemic clamp technique. At baseline, all of these responses were diminished and occurred at much lower blood glucose levels in the diabetic subjects than in the non-diabetic subjects. After three months of intensive diabetes control during which the principal aim was complete avoidance of any hypoglycaemia, the differences between diabetic and non-diabetic subjects were minimal. In particular, diabetic subjects showed cognitive dysfunction at higher blood glucose levels than before the intervention, presumably reflecting reverse adaptation [65,67].

Mitrakou *et al* obtained similar results in an elegant, opportunistic study in patients with insulinomas, who suffer recurrent or chronic hypoglycaemia prior to surgical cure.

Using a similar stepped glycaemic clamp, it was shown that prior to surgery patients could maintain cognitive performance at blood glucose levels as low as 2.3 mmol/l, and that this effect was entirely abolished after surgical resection (Figure 2.4) [64].

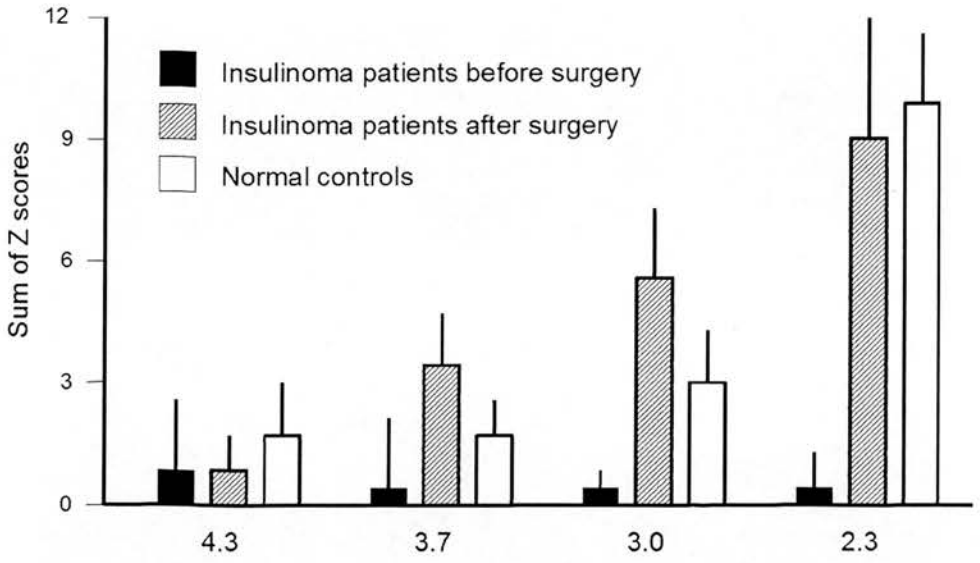


**Figure 2.4.** Cognitive function during progressive hypoglycaemia in normal subjects, and subjects before and after removal of insulinomas. Higher $z$-scores represent worse cognitive function. Based on (but not reproduced from) graph in Mitrakou *et al* [64].

### 2.5.5 Clinical significance of adaptation of cognitive function to hypoglycaemia

People with insulin-treated diabetes commonly develop impaired awareness of hypoglycaemia, as discussed previously in section 1.6. This manifests as a lowering of the blood glucose levels at which symptomatic and hormonal responses are initiated [85,86,87,88]. If the glucose levels for cognitive function were to remain unchanged, then cognitive impairment would develop before symptomatic awareness of hypoglycaemia; this hypothetical re-ordering of the normal hierarchy is shown in Figure 2.5. In conjunction with impaired counterregulation, this re-ordering could underlie the higher incidence of severe hypoglycaemia that is associated with strict glycaemic control and diabetes of long duration [89]. In effect, it would mean that the diabetic person would already be confused due to neuroglycopenia by the time they realised they were hypoglycaemic – perhaps too confused to be able to successfully treat themselves.

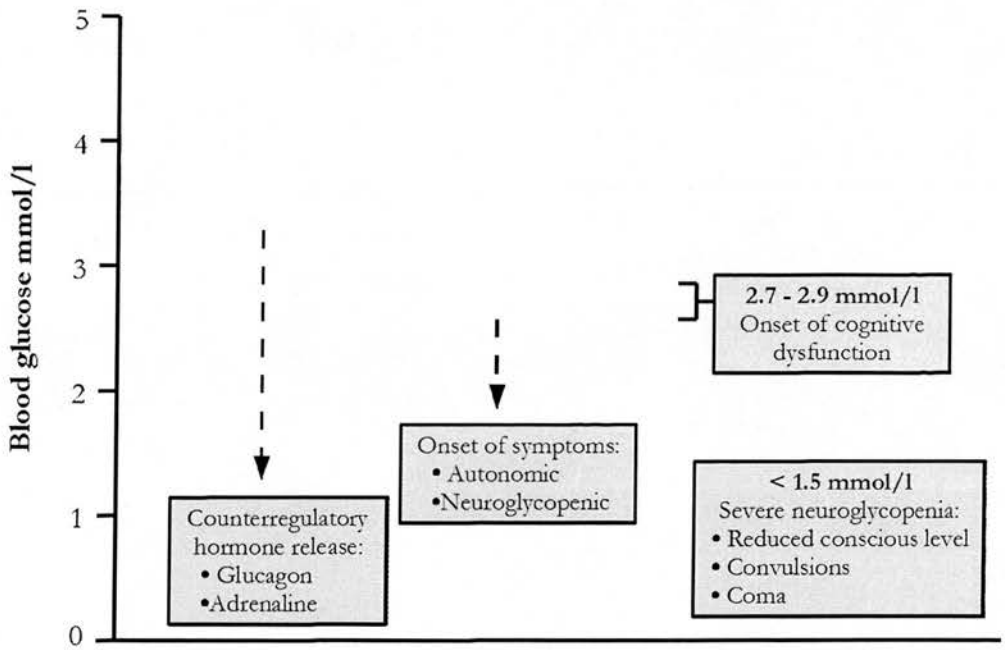


**Figure 2.5.** Hypothetical change in blood glucose levels in impaired hypoglycaemia awareness.

As discussed previously, there is good evidence that the brain does adapt to recurrent hypoglycaemia. However, in the three studies where glycaemic thresholds were measured in diabetic subjects with impaired hypoglycaemia awareness, statistically-significant cognitive impairment occurred at a similar or higher blood glucose level than

the symptomatic and counterregulatory responses [64,67,69]. It therefore appears that cerebral adaptation to recurrent hypoglycaemia cannot match the decline in symptomatic and counterregulatory defences, with the result that the first manifestation of hypoglycaemia in the unaware patient is confusion.

### 2.5.6 Conclusions regarding cerebral adaptation to hypoglycaemia

The results of interventional studies showing longitudinal changes in glycaemic thresholds are particularly persuasive [16,64,65,67], and overall it seems clear that cerebral adaptation to recurrent hypoglycaemia is a real phenomenon. The evidence so far cited indicates that adaptation does not occur during 60 minutes [24,26], may occur during 90 minutes [73], probably occurs during 150 min [74], and does occur during 56 hours [16] of hypoglycaemia. Also, following two to three hours of hypoglycaemia, cognitive adaptation to subsequent hypoglycaemia may occur within two hours [75], does occur within seven hours [76] and lasts between 24 and 48 hours [45,62]. These results suggest that a very prolonged hypoglycaemic stimulus is not required, but the adaptive process itself may take some time. The mechanism of adaptation may be an increase in brain glucose uptake capacity [16,80]. By contrast, studies in rats indicate that several days of hypoglycaemia are required to induce changes in brain glucose transport via upregulation of the GLUT1 transporter [78,79,90], and if this is also true of humans, there may be another mechanism for acute adaptation such as increased production of alternative brain fuels. However, neither the existence nor the nature of a second adaptive mechanism is clear.

Nonetheless, impaired awareness of hypoglycaemia is clearly associated with severe disabling hypoglycaemia [89], and cognitive adaptation is not sufficient to protect against this. This appears to be because the autonomic symptoms are lost or diminished to a much greater extent [64,67].

## 2.6 Support of cognitive function during hypoglycaemia

Under normal circumstances, the brain obtains almost all its metabolic energy from oxidation of glucose, so hypoglycaemia causes an acute energy deficiency. The brain is also capable of generating energy from lactate, ketones and alanine, and intravenous infusions of these substances attenuate cognitive dysfunction during hypoglycaemia [61,68,91,92]. However, it is not clear how this could be useful in clinical practice, as intravenous infusion is not a practical intervention in daily life, and the presence of the first two in physiological circumstances is a result of dangerous metabolic derangement.

More interest has been focused on interventions to increase cerebral glucose utilization. Administration of acetazolamide has been shown to increase cerebral blood flow (CBF), resulting in attenuated symptomatic and counterregulatory hormonal responses to hypoglycaemia [44]. No effect was seen on cognitive function as measured by P300 latency, although this does not rule out beneficial effects on other cognitive measures, and more work with acetazolamide may be merited. Kerr *et al* administered caffeine during or prior to hypoglycaemia, resulting in reduced CBF [93,94]. They documented heightened symptomatic awareness of hypoglycaemia with neutral or detrimental effects on cognitive function, suggesting that caffeine simply resulted in more severe neuroglycopenia for a given blood glucose level, and it is therefore unlikely that caffeine can be of value as a therapeutic option.

Insulin promotes uptake of glucose into tissues, and it is logical to question whether hyperinsulinaemia might improve brain function during hypoglycaemia, through effects on glucose transport rather than blood flow. Brain glucose uptake was considered insulin-independent, but has been re-examined in recent years using positron emission tomography and magnetic resonance spectroscopy. Two studies reported no effect of hyperinsulinaemia on brain glucose utilization [4,5]. Another study reported a small increase in glucose uptake with physiological, compared with sub-physiological, plasma insulin concentrations, but this finding may not be relevant to diabetes, where hypoglycaemia is associated with hyperinsulinaemia [7]. Regarding cognitive performance, two studies found no effect of hyperinsulinaemia [33,63]. Another

reported some improvement during euglycaemia with a very high insulin infusion rate (15 mU $kg^{-1}$ $min^{-1}$), but only at one time-point, raising the possibility of type 1 statistical error [95].

The GABA-inhibitor modafinil is a stimulant usually used to treat narcolepsy. Smith *et al* administered modafinil prior to experimental hypoglycaemia, and reported that it was associated with a reduction in the number of incorrect scores on the Stroop colour-word task (P=0.005), and an improvement in the accuracy of a decision reaction time task (P=0.04) [96]. These are not the standard measures for these tasks and were not obviously pre-specified, raising the possibility of a type 1 statistical error due to multiple comparisons. The same group examined modulation of cerebral K-ATP channels using diazoxide and glibenclamide [59]. The estimated blood glucose threshold for deterioration of choice reaction time was 2.5 mmol/l following diazoxide, and 3.0 mmol/l following glibenclamide (P=0.002 for comparison); however, no significant effects were demonstrated against placebo, so it is unclear whether glibenclamide was beneficial or diazoxide detrimental.

Overall, no intervention has shown clear potential to prevent cognitive deterioration during hypoglycaemia, and the odds may be against development of such a therapy. As illustrated in Figure 2.4, patients exposed to prolonged hypoglycaemia compensate to an extraordinary degree, and there may be little scope for further compensation for a fundamental deficit in fuel supply. The underlying problem in impaired hypoglycaemia awareness is altered thresholds for symptoms and counterregulation, and strategies to restore these may hold more therapeutic potential.

## 2.7 Long-term effects of hypoglycaemia

Profound, protracted hypoglycaemia can cause permanent brain damage or death. Post-mortem studies [97,98,99] and neuroimaging studies [100,101,102,103,104] of individuals who had suffered fatal hypoglycaemia have revealed areas of cortical injury, particularly in the frontal lobes and hippocampus. Although such catastrophic hypoglycaemia is rare, it is possible that recurrent hypoglycaemic episodes which are less severe but nonetheless disabling may cause subclinical brain damage, leading to cognitive impairment over time. Anecdotal reports of diabetic patients who have developed mental difficulties have attributed this to recurrent severe hypoglycaemia [105].

Permanent functional brain abnormalities have been associated with recurrent hypoglycaemia, including electroencephalographic abnormalities [106,107,108]. Cerebral blood flow is redistributed during acute hypoglycaemia, with increased frontal lobe perfusion [109], and this pattern appears to become permanent in diabetic patients who have suffered recurrent severe hypoglycaemia [110] or impaired hypoglycaemia awareness [111]. Structural changes associated with hypoglycaemia are less apparent. Neuroimaging has demonstrated abnormalities associated with type 1 diabetes, including leukoaraiosis, small periventricular lesions representing foci of ischaemia [112,113]. This has led to the concept of "diabetic encephalopathy", to which vasculopathy, hyperglycaemia and hypoglycaemia may contribute. However, as these conditions co-exist, no single pathogenetic mechanism can be deduced. A recent study from our centre found a strong association between leukoaraiosis and retinopathy but not with hypoglycaemia, suggesting that leukoaraiosis represents a microvascular complication of hyperglycaemia [114].

The long-term effects of severe hypoglycaemia on cognitive function are not fully defined. A number of studies have reported intellectual disadvantage in patients with histories of severe hypoglycaemia [115,116,117,118,119], but this association may arise simply because less able patients manage their diabetes less effectively, and so suffer more hypoglycaemia [119]. In a study from Edinburgh, current IQ was measured and premorbid IQ was estimated in diabetic patients, whose history of severe hypoglycaemia

was also determined [120]. A highly significant decline in IQ was seen in patients who had suffered at least five episodes of severe hypoglycaemia, with no significant decline in patients with no history of severe hypoglycaemia (Figure 2.6); a study from Nottingham using a similar design reported similar results [121]. However, there was no significant difference in the change in IQ. A limitation is the estimation of pre-morbid IQ, which was based on National Adult Reading Test scores. Performance on this test correlates highly with Wechsler Adult Intelligence Scales-Revised (WAIS-R) IQ, is resistant to the effects of organic brain damage, and so may indicate 'best ever' intelligence [122]. Nonetheless, the correlation between NART and WAIS-R performance is imperfect, and it is therefore possible that NART estimates of intelligence will tend to regress closer to the mean than WAIS-R scores in the same individuals, reducing apparent differences between patient groups. The data are suggestive, but a prospective study design would be more robust.

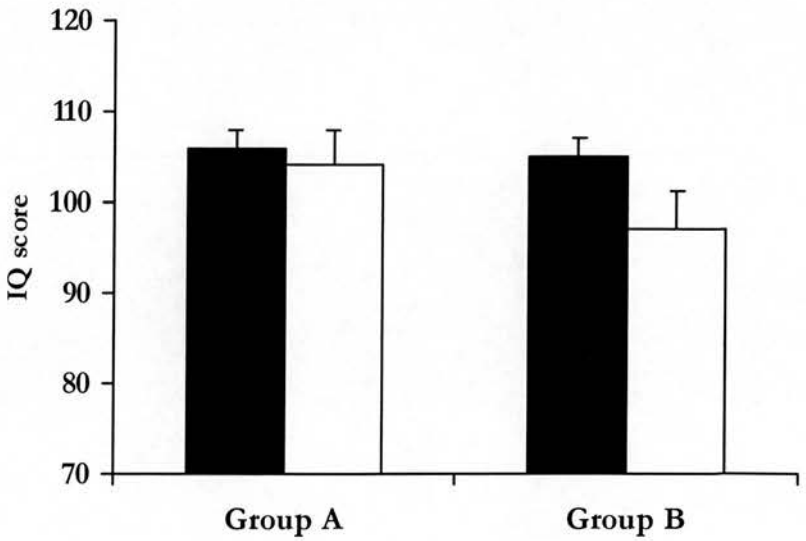


**Figure 2.6**. Estimated pre-morbid and current IQ in diabetic patients with no previous severe hypoglycaemia (group A) and five or more severe glycaemic episodes (group B). Comparison for Group A is non-significant, comparison for group B is significant at $p<0.001$. From [120].

The major prospective trial of type 1 diabetes is the Diabetes Control and Complications Trial. Patients underwent detailed cognitive function testing at entry, and subsequently, depending when they were enrolled, at one or more of 2, 5, 7 and 9 years

later, amounting to median follow-up of 6.5 years when the trial finished. No association between repeated episodes of severe hypoglycaemia and cognitive decline was demonstrated [123,124]. Follow-up data from the DCCT have now been published, on 1144 patients undergoing detailed cognitive function testing 18 years after the start of the trial [125]. There were no differences between patients originally allocated to intensive or conventional insulin therapy, and no association between frequency of severe hypoglycaemia and cognitive change. The only statistically significant association was between poor glycaemic control ($HbA_{1c}$>8.8%) and decline in motor and psychomotor performance. This is by far the largest prospectively-collected dataset that exists on this topic, and it provides further support for tight glycaemic control, suggesting that it should prevent cognitive decline due to hyperglycaemia, without causing cognitive dysfunction due to hypoglycaemia. The DCCT population is known to be somewhat atypical of the general diabetes population, with a low rate of severe hypoglycaemia [126]. However, a specific research question was whether individuals with five or more episodes of severe hypoglycaemia showed cognitive impairment, and at the end of the 18-year follow-up period, there were 66 such individuals, which provides reasonable power to detect an effect. The DCCT findings cannot exclude the possibility that more frequent severe hypoglycaemia causes harm. They also cannot be translated to the entire diabetic population, who often experience hypoglycaemia in the context of psychosocial problems and erratic lifestyle [127], and vascular disease may increase the risk of hypoglycaemic brain damage [128]. Trials randomising patients to non-intensive treatment would now be considered unethical, and it is unlikely that there will ever be definitive answers.

## 2.8 Hypoglycaemia and cognitive function in children

Acute hypoglycaemia causes cognitive impairment in children [129], but has been studied less extensively than in adults. Matyka *et al* showed that, while nocturnal hypoglycaemia was frequent in children, cognitive function was unimpaired the next morning [130]. However, concern has been expressed about repeated exposure of the immature brain to hypoglycaemia [131]. Retrospective studies have reported cognitive disadvantage in later life in children with early-onset diabetes (age less than 4-5 years) by comparison with children who developed diabetes at an older age [132,133,134]. Although one possible cause is recurrent hypoglycaemia, hyperglycaemic brain disease or disruption of education and psychosocial development at a susceptible age could equally explain these findings.

Several retrospective and prospective studies have reported that hypoglycaemia, rather than some other aspect of diabetes, is correlated with educational and intellectual disadvantage among children with diabetes [135,136,137,138,139,140]. Although these studies were individually too small or too short to provide reliable results, and were often prone to type 1 statistical errors, the association appears to be consistent. A prospective Australian study has compared 90 children with type 1 diabetes with matched non-diabetic controls shortly after diagnosis, and again after two and six years [141]. No differences in intellectual function were observed at baseline, but by six years a clear disadvantage in the diabetic children had emerged, particularly in verbal IQ. In regression analysis, hypoglycaemic seizure history was a significant predictor, and explained 3% of variance in full-scale IQ. This is not a large effect, and the major influence of diabetes may have been hyperglycaemia or psychosocial disruption. Non-interventional studies cannot resolve the question of whether hypoglycaemia is the cause or the effect of lower intelligence. Nonetheless, it is probably appropriate to err on the side of caution by attempting to avoid all severe hypoglycaemic episodes, although it is difficult to achieve this at the same time as achieving good glycaemic control.

## 2.9 Memory function during hypoglycaemia

Discussion of memory function during hypoglycaemia has been omitted in the preceding sections, as this is a main subject of this thesis. Recently, memory has been examined in depth in Edinburgh. Previously there was relatively little work on the subject.

### 2.9.1 Older studies of memory and hypoglycaemia

Holmes *et al* studied 12 students with type 1 diabetes at three levels of non-arterialized venous blood glucose: 3.3, 6.1 and 16.7 mmol/l [10]. The memory tests used were (1) the *Rey Auditory Verbal Learning Test (AVLT)*, in which a list of 15 words is read out to the subject, who attempts to repeat them immediately; the score is the number of words recalled, and the procedure is repeated for a total of five trials with the same word list; (2) *digit span*, in which progressively longer sequences of digits are read out to the subject, who attempts to repeat them immediately; the score is the number of digits in the longest sequence the subject is able to repeat. Performance on each test did not differ at different blood glucose concentrations. From today's perspective this is not surprising, as a venous blood glucose concentration of 3.3 mmol/l (roughly equivalent to an arterial or arterialized venous concentration of 3.6-4.0 mmol/l) is well above the thresholds estimated for cognitive dysfunction in non-diabetic subjects (see section 2.3). Furthermore, diabetic subjects may sustain cognitive function at lower blood glucose levels due to adaptation resulting from previous hypoglycaemia (see section 2.5). The study did not include a euglycaemia control study, although as the order of glycaemic condition varied and was counterbalanced between subjects, this is a relatively minor criticism.

Pramming *et al* also used digit span to measure memory function in 16 subjects with type 1 diabetes at target venous blood glucose concentrations of 6.3, 2.9 and 1.8 mmol/l [19]. Their method of statistical analysis was unusual. For each subject, scores on the digit span test at the different glycaemic levels were ranked in order, and a total score at each glycaemic level was created by summing the ranks for all subjects at that level. This was effectively a variant of conventional rank-order statistical tests. A change in

rank scores indicating deteriorating performance was seen with target blood glucose levels of 2.9 and 1.8 mmol/l. A sham euglycaemia control study was included, in which no significant changes in memory performance were reported, but the effects of hypoglycaemia were not calculated on the basis of difference between hypoglycaemia and euglycaemia studies, and thus the data from the control arm were misused by today's standards. Blood glucose concentrations were not well-controlled, with sometimes large differences between achieved and target concentrations.

Harrad *et al* measured digit span performance at target venous blood glucose concentrations of 2.5 and 1.5 mmol/l in 11 subjects, of whom 5 had type 1 diabetes and were 6 healthy [47]. No changes were seen in digit span performance. Blood glucose control was extremely poor, such that the study only really achieved blood glucose levels somewhere below 3 mmol/l at each target level. Widom & Simonson tested digit span performance and word recall in 10 non-diabetic subjects, 8 subjects with type 1 diabetes and good glycaemic control and 9 subjects with type 1 diabetes and poor glycaemic control [21]. The word recall test involved reading out three words to subjects, and testing recall after five minutes. They attempted to define glycaemic thresholds for deterioration on these and other cognitive tests. No deterioration was seen in digit span and word recall performance despite achieving a nadir arterialized venous glucose concentration of 2.2 mmol/l. Actual scores were not reported, but it is likely that the word recall test was too easy to be a sensitive measure of cognitive decline.

Wirsén *et al* studied cognitive performance at an arterialized-venous blood glucose concentration of 2.0 mmol/l in 10 subjects with type 1 diabetes and 12 non-diabetic subjects [17]. Memory tests included story recall (no citation was provided and this may have been a bespoke test) and digit span with forwards and backwards recall. Significant impairment on all these tests during hypoglycaemia was seen in all subjects combined. There also seemed to be less effect of hypoglycaemia on non-diabetic subjects for the forward digit span task. The study was well-designed apart from the absence of a euglycaemic control session.

In summary, the older studies of memory function reported variable results, but were generally scientifically weak. The only study of nearly satisfactory quality showed a deterioration in digit and story recall during hypoglycaemia [17].

### 2.9.2 Edinburgh studies of memory and hypoglycaemia

Sommerfield *et al* studied memory performance in 16 non-diabetic subjects and 16 people with type 1 diabetes during euglycaemia (4.5 mmol/l) and hypoglycaemia (2.5 mmol/l) [28,30,142]. Cognitive tests used were in the following domains:

*Verbal memory*: the Auditory Verbal Learning Test, immediate and delayed, as described above, and the Logical Memory Test, immediate and delayed, from the Wechsler memory scales [143]. In the Logical Memory Test, a short story is read and points are scored for recollection of detail and thematic elements.
*Visual memory*: the Visual Reproduction Test, immediate and delayed, and the Benton Visual Retention Test [144]. In both tests, progressively more complex geometric designs are briefly shown, and must be reproduced from memory.
*Working memory*: the Working Digit Span and Letter/Number Sequencing tests from the Wechsler memory scales [143], and the Validation Span and Four Term Order tests from Kyllonen's battery [145]. The first two tests require the subject to listen to sequences of numbers or numbers and letters, and then to recite them in numerical and alphabetical order. The Validation Span test requires subjects to remember digits while simultaneously solving arithmetical problems to prevent rehearsal. The Four Term Order test is best illustrated by example: "The animals come after the furniture; the horse comes before the dog; the desk does not come before the rug." Eight response alternatives are displayed on a card, and the subject would score correctly by choosing the sequence "rug-desk-horse-dog."
*General cognition*: the Digit Symbol Substitution and Trail-Making B tests.

Performance on both Digit Symbol Substitution and Trail Making B declined significantly in both groups. Table 2.4 gives performance and statistical comparisons for euglycaemia and hypoglycaemia. Memory performance was significantly impaired under hypoglycaemic conditions for all tests except Wechsler Visual Reproduction –

immediate, and Digit Span Forward in diabetic subjects. The average effect size ($\eta^2$) was approximately 0.5, which is a large effect; particularly large effect sizes ($\eta^2>0.6$) were seen for delayed memory and some working memory tests.

| *Memory domain* | *Subtest* | *Diabetic* | | | | *Non-Diabetic* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Eu* | *Hypo* | *P* | $\eta^2$ | *Eu* | *Hypo* | *P* | $\eta^2$ |
| **Immediate verbal** | Logical Memory | 26.8 (4.8) | 21.3 (6.9) | .008 | .41 | 27.7 (5.9) | 24.4 (5.0) | .004 | .45 |
| | AVLT | 39.4 (7.7) | 34.0 (5.1) | .002 | .49 | 43.6 (7.0) | 33.9 (7.7) | .001 | .53 |
| **Immediate visual** | Benton VRT | 6.5 (1.7) | 4.7 (1.5) | .007 | .42 | 6.3 (1.7) | 5.1 (1.6) | .041 | .26 |
| | Wechsler VRT | 81.3 (7.2) | 78.2 (9.5) | .093 | .19 | 85.6 (8.3) | 81.7 (8.0) | .059 | .23 |
| **Delayed verbal** | Logical Memory | 13.6 (2.1) | 6.1 (3.7) | .0001 | .83 | 15.1 (4.0) | 8.1 (3.9) | .001 | .58 |
| | AVLT | 9.1 (1.9) | 5.3 (1.9) | .0001 | .68 | 10.3 (2.1) | 4.5 (1.6) | <.0001 | .87 |
| **Delayed visual** | Wechsler VRT | 15.9 (8.7) | 7.1 (7.3) | .002 | .52 | 21.5 (16.5) | 7.6 (5.5) | .001 | .57 |
| **Working memory** | Validation Span | 20.7 (2.3) | 14.9 (2.2) | .0001 | .92 | 22.0 (2.3) | 13.4 (2.5) | <.0001 | .94 |
| | Digit Span Forward | 9.6 (2.4) | 8.6 (1.4) | .06 | .23 | 10.2 (2.4) | 9.3 (1.7) | .042 | .26 |
| | Digit Span Backward | 8.3 (1.9) | 7.2 (1.2) | .02 | .33 | 8.8 (1.9) | 6.8 (1.2) | <.0001 | .67 |
| | Letter/Number Sequencing | 11.8 (1.9) | 9.7 (1.6) | .0001 | .57 | 13.1 (3.1) | 10.5 (1.8) | .008 | .40 |

**Table 2.4**. Performance on memory tests during eu- and hypoglycaemia in type 1 diabetic and non-diabetic subjects in [28,30].

A remarkable effect of hypoglycaemia was seen on performance on the Four Term Order test. Results from diabetic and non-diabetic subjects for this test were combined, and do not appear in Table 2.4. Mean (SD) scores were euglycaemia: 12.4 (4.3), hypoglycaemia: 3.7 (2.3), $P<0.001$, $\eta^2=0.86$. Each subject attempted 24 items in the Four Term Order test, and with eight choices for each item, the expected score from random answering is 3.0. Thus, the scores during hypoglycaemia were reduced almost to chance level, suggesting that working memory was obliterated [142]. Working memory correlates very strongly with other measures of general fluid intelligence [146], and so this result suggests that complex reasoning ability should also be crippled by moderate hypoglycaemia. However, the result should be considered in context. Performance on the four other tests of working memory in Table 2.4 was certainly not obliterated. The Four Term Order test is hard, even for able subjects during euglycaemia, and the disparate results perhaps reflect the concept of cognitive 'spare capacity'. If a test is easy under normal conditions, then there is spare capacity, and

hypoglycaemia (or other interference) may first use up this spare capacity such that the test may be completed by dint of increased concentration or mental effort. On the other hand, if a subject is only just able to complete a test under normal conditions then relatively minor interference may render the test impossible.

Previous studies have shown clear evidence of complex reasoning ability during moderate hypoglycaemia. For example, McAulay *et al* found that non-verbal intelligence, as assessed by Raven's Progressive Matrices, was not affected during hypoglycaemia at 2.5 mmol/l [38]. In Raven's Progressive Matrices, each question presents a series of geometric shapes governed by simple rules that must be deduced. One shape is missing, and the subject must identify the missing shape from eight candidates. The nature of this task makes it clear that working memory must be involved, and so working memory cannot be completely obliterated by moderate hypoglycaemia. However, in keeping with the concept of spare capacity, it is notable that in the study by McAulay *et al*, baseline (i.e. euglycaemic) performance was at a high level (around 80% correct), and it is possible that the logical reasoning task was too easy to be discriminative.

Working memory is a specialized form of short-term memory that is closely associated with intelligence. The verbal and visual memory tests utilized what would more conventionally be considered short- and long-term memory, and showed significant deterioration. The practical relevance of these studies has limitations. Learning and recall-testing took place during the same period of hypoglycaemia, lasting approximately one hour. This narrow separation of learning and recall must occur relatively infrequently in real life – intuitively, more common situations would be an extended learning period (e.g. when studying for examinations) or a longer interval between learning and recall (e.g. when sitting the examination, or remembering an acquaintance's name). The examination of the effects of hypoglycaemia on different memory processes forms the major part of this thesis, as described in Chapter 3.

## 2.10 References


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# Chapter 3

# Research Aims

## 3.1 Research Aims

Over the previous three decades, a large body of research into the effects of hypoglycaemia on cognitive function has been created in Edinburgh. Many of the previous studies have been described or cited in Chapters 1 & 2. The research described in this thesis was conceived with the aim of continuing where previous studies had left off.

### 3.1.1 Hypoglycaemia and memory function

Sommerfield and colleagues examined the effects of acute, moderate hypoglycaemia (2.5 mmol/l) on various aspects of memory function, as described in section 2.9.2 [1,2,3]. In these studies, both learning and recall testing took place during the same episode of hypoglycaemia, with a maximum interval of 60 minutes. In daily life, the interval between acquiring information and needing to remember it may be much greater, and it is unlikely under such circumstances that both would occur during separate episodes of hypoglycaemia.

Study 1 (in Chapter 4) examines the possibility that an episode of hypoglycaemia occurring shortly after learning may disrupt the long-term storage of memories, i.e. that hypoglycaemia affects the consolidation of memory. As will be discussed in that chapter, there is evidence indicating that the hippocampus is a critical structure in the formation of long-term memories, and that it is particularly sensitive to the acute effects of hypoglycaemia. Functional magnetic resonance imaging is a method for examining regional brain activation that has recently become available, and is utilized in Study 1 to

try to determine whether hippocampal dysfunction explains any memory impairment following hypoglycaemia.

Study 2 (Chapter 5) examines the effect of (i) an episode of hypoglycaemia occurring during learning, with recall taking place later during euglycaemia, and (ii) an episode of hypoglycaemia occurring during recall, learning having taken place earlier during euglycaemia. The first manipulation is intended to mimic the situation where a person with diabetes finds themselves to be hypoglycaemic while studying (for example) – should they review all material they have read recently, in case it has not registered? The second manipulation is intended to mimic the discovery of hypoglycaemia during an examination (for example) – will their recent memory for previously learned material have been deficient?

Study 2 also includes the development of a novel test of prospective memory. Many of the memory tests that have been utilized in hypoglycaemia studies seem to reflect the role of memory in daily life poorly – it is rare that one needs to learn a list of words and immediately recall it, for example. Also, in most memory tests, questions are explicitly asked, whereas in daily life a more common need is to remember to do something.

### 3.1.2 Hypoglycaemia and non-verbal intelligence

McAulay and colleagues found no statistically significant effect of moderate hypoglycaemia on performance on a test of abstract non-verbal intelligence (Raven's Progressive Matrices), although attentional function was impaired [4,5]. This was unexpected, as it has often been argued that higher-level cognitive functioning is more sensitive to hypoglycaemia than lower-level tests with a large motor component (see section 2.2.4). One possible explanation is that Raven's Progressive Matrices is too easy a test for the often very intellectually able subjects who participate in research studies. Study 3 examines this by repeating the study design with a harder version of the same test.

### 3.1.3 Speed versus Accuracy of Cognitive Function

As discussed in section 2.2.2, cognitive function tests in hypoglycaemia studies usually measure speed of completion of tasks that are intrinsically easy. During hypoglycaemia (or any other adverse state), one strategy may be to maintain speed at the expense of accuracy – for example, to guess more. In previous studies (and, as it would prove, in Studies 1 & 2 here), the number of errors made during reaction time, Trail Making and Digit Symbol Substitution tests are too few for accuracy to be measured. To try to answer the question of whether speed or accuracy are favoured during hypoglycaemia, Study 3 also includes a maze task scored for both.

### 3.1.4 Symptoms of Hyperglycaemia

The effects of acute hyperglycaemia have received very little attention, though clinical experience is that it causes significant symptoms and may affect mental performance. Sommerfield *et al* recently produced experimental evidence for hyperglycaemic cognitive dysfunction [6], raising the possibility that other aspects of hyperglycaemia may also have parallels with hypoglycaemia. Study 4 is designed to identify the symptoms of hyperglycaemia, and to examine them using factor analysis in the same way as was done for hypoglycaemia symptoms, as described in section 1.5.

## 3.2 References


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# Chapter 4

# Effects of Hypoglycaemia on Memory Consolidation

## 4.1 Introduction

Memory has been conceptualized as composed of three main subsystems: sensory memory, short-term memory, and long-term memory [1]. Sensory memory is the brief retention of an auditory or visual impression after the stimulus has been removed. Short-term memory can hold a limited number of items, with a duration measured in seconds. Long-term memory is durable, with no quantifiable capacity limits. The conversion of short- to long-term memory is known as consolidation. Little is known about the neurological basis of consolidation, although it is probable that many different areas of the brain are involved in long-term storage. However, the hippocampus and adjacent medial temporal lobe structures appear to be critical to the formation of long-term memory. Evidence for this comes from individuals who have sustained focal damage to the hippocampus and have developed near-total inability to form new long-term memories, while retaining intact short-term memory [2,3,4,5]. There have been case reports of individuals who have developed amnesia following severe hypoglycaemia, in whom frontal and hippocampal lesions were identified on MRI scans [6,7]. Post-mortem examinations of individuals who have died following catastrophic hypoglycaemia have revealed areas of necrosis in the frontal cortices and hippocampus, with relative sparing of the hindbrain [8,9,10,11]. It appears that the frontal lobes and hippocampal region are particularly sensitive to hypoglycaemia, and this may explain the mechanism by which moderate hypoglycaemia disrupts aspects of memory.

In previous studies of memory during hypoglycaemia, stimulus presentation and recall testing both took place during the experimental condition (hypoglycaemia or euglycaemia) [12,13,14,15,16,17,18,19]. Although these studies demonstrated memory impairments, they could not disentangle the effects of hypoglycaemia on acquisition

(learning), recall and consolidation. In a practical context, the possibility that hypoglycaemia impairs information acquisition and recall may not be very surprising: no student with diabetes would wish either to study for, or to sit, an examination while hypoglycaemic. The possibility that consolidation is disrupted by hypoglycaemia is important, because it is not obvious but is potentially of considerable practical relevance. Consolidation is the fundamental process by which new information becomes part of our knowledge stores. Consolidation may take some time, and if it were sensitive to hypoglycaemia then it would follow that hypoglycaemia could impair memory formation for events that occurred *prior* to the onset of hypoglycaemia.

The present study was designed to determine whether a period of hypoglycaemia immediately following the learning process can disrupt consolidation and impair subsequent recall. To examine the mechanisms by which hypoglycaemia might exert an effect on memory, functional magnetic resonance imaging (fMRI) was used to inspect regional brain activation during recall.

fMRI uses the blood oxygen level dependent (BOLD) signal, which exploits a natural difference in the magnetic properties of oxy-haemoglobin and deoxy-haemoglobin [20]. When a subject is exposed to a stimulus or task, neuronal activation in related brain regions increases, and this is followed after 10-12 seconds by local vasodilatation [21,22]. Although local oxygen use increases, the hyperaemic response more than compensates, so that the concentration of deoxy-haemoglobin is reduced and the BOLD signal from those regions is increased. A three-dimensional map of brain activation can thus be generated. The speed of acquisition of fMRI allows activation maps to be generated every few seconds, and so maps can be obtained for each task in a battery, between which the experimental condition can be varied. Other techniques, such as positron-emission tomography (PET), require longer periods for image acquisition, and a block design is required, whereby an average activation map is obtained over the course of several tasks during which the experimental condition is held constant. The advent of fMRI has permitted study of the activation of brain structures during very specific memory tasks. Activation of the medial temporal lobe structures (including the hippocampus) has been consistently associated with successful encoding and retrieval of episodic memories [23]. The pre-frontal cortex is also profoundly involved in memory

processes, although in fMRI studies pre-frontal activation has been more consistently associated with retrieval effort rather than successful retrieval of episodic memories [23].

## 4.2 Pilot Study

### 4.2.1 Pilot Study – Introduction

A pilot study was undertaken to develop and test an appropriate memory task for the main study. The learning phase of the task was to be applied during the euglycaemic phase of the glucose clamp, and the recall (test) phase in an MRI scanner following an hour of euglycaemia or hypoglycaemia.

There were certain important restrictions imposed by the use of MRI. MRI scanning is extremely noisy, and so it is hard to use verbal communication or auditory stimuli. Subjects must lie prone and still in the scanner, excluding the use of a computer keyboard. Response options are therefore generally restricted to the subject pressing buttons with hands held at either side, and since the subject cannot look at the buttons, only a binary response (left or right hand) is practical. Consequently, none of the established memory tasks that had been used previously in hypoglycaemia studies could be used, as these all required verbal or written responses, usually to verbal questions. A further restriction was the requirement for fixed timing of the memory task, so that the fMRI signal could be correlated with the onset of the memory retrieval effort. Previous fMRI studies of memory have used visual recognition tasks [23]. Typically this paradigm requires subjects to view a series of images, and later to view the same images mixed with new images, and identify each as old or new. This only requires subjects to look at a screen while in the MRI scanner, and identify stimuli as new or old using left and right hand button presses. The time of presentation of each test stimulus is known, and hence the onset of memory retrieval can be linked to simultaneous fMRI acquisition by appropriate computer software.

### 4.2.2 Pilot Study – Methods

In the present study, two forms of visual stimuli were used: faces and words. For the face stimuli, digitized photographs of a Caucasian student population were used, and scaled so that the head occupied 80% of the frame. Pictures were taken without jewellery or spectacles. For the word stimuli, words with frequencies between 20 and 30

in the series from Kucera & Francis [24] were used. Two batteries were created, each containing 40 faces (20 male, 20 female) followed by 50 words.

These recognition memory tasks were piloted on 12 non-diabetic volunteers, predominantly colleagues at the Royal Infirmary of Edinburgh and the University of Edinburgh. Pilot studies took place in empty consulting rooms in the Royal Infirmary, in silence, with instructions provided on a computer screen. Stimuli were presented to volunteers on a computer screen positioned at arms length, and volunteers were asked to attempt to memorize these. To improve retention, subjects were asked to rate each word or face for its attractiveness, although these data were not analysed.

Recognition was tested after an interval of one hour. For the face recognition task, subjects viewed a series of 90 faces, of which 40 had been seen before (targets) and 50 were new (decoys). For the word recognition task, there were 140 stimuli, comprising 50 targets and 90 decoys. Subjects were required to identify targets by pressing a left-hand or right-hand computer key.

Individual responses were classified as 'Correct Hits', 'Incorrect Hits' (i.e. subject stated that they had seen the face or word previously when in fact they had not), 'Correct Rejections' and 'Incorrect Rejections'. The numbers of responses in each category were calculated for each subject. However, none of these provides an adequate measure of memory performance, as they may be influenced by strategy. If memory performance were judged on 'Correct Hits' alone, then subjects responding 'Yes' every time would score full marks. If performance were judged on 'Correct Hits' + 'Correct Rejections' then a tendency to answer 'No' would give higher marks, as there were more decoys than targets. Instead, stimuli correctly identified were converted into an adjusted score, weighted for the true frequency of targets and decoys so that the chance score was 50. The equation for this is $(H/T+R/D)x50$, where H is the number of correct hits, R is correct rejections, T is targets and D is decoys.

### 4.2.3 Pilot Study – Results and Conclusions

Mean corrected scores of 71% and 75% were obtained for the face and word tasks respectively. The expected minimum score (i.e. that which would be obtained by random answering) is 50%. These mean scores appeared to fall appropriately between floor (50%) and ceiling (100%) limits, indicating that the visual recognition tasks were not excessively hard or easy. There were no individual test items that were excessively easy or hard (i.e. no words or faces that were consistently recognised or not recognised by most subjects). Accordingly, the memory task was felt appropriate to be used in the main study.

## 4.3 Main Study - Methods

### 4.3.1 Ethics

The study was approved by the local research ethics committee, and all subjects provided written and informed consent to participate.

### 4.3.2 Subjects

For the main study, sixteen right-handed non-diabetic adults (9 male) for whom English was a first language were recruited. Subjects with active medical conditions or previous histories of seizure, cerebral injury or any other contra-indication to experimental hypoglycaemia were excluded. All subjects completed the National Adult Reading Test (NART) at the start of their first session. In the NART, the subject is presented with a list of words that are used very infrequently in everyday spoken English, and is asked to read them aloud. Pronunciation is scored with reference to an audio tape of correct pronunciation. Performance on this test has been shown to correlate very highly with best-ever Wechsler Adult Intelligence Test scores [25]. The mean score on the National Adult Reading Test was 40, approximating to a WAIS-R Full Scale IQ score of 118. Subject details are given in Table 4.1.

| | |
|---|---|
| **Sex** | 9 male: 7 female |
| **Age** | 27.5 (20-44) |
| **BMI** | 22.9 (20.1-27.7) |
| **NART** | 39.6 (28-49) |

**Table 4.1.** Subject details. Mean and range are provided for age, BMI and NART score; numbers male and female are given for gender.

### 4.3.3 Study outline and glucose clamp procedure

Subjects underwent two study sessions (hypoglycaemia and euglycaemia) separated by at least two weeks. The study design is illustrated in Figure 4.1.

Subjects fasted overnight and refrained from drinks containing caffeine or other stimulants. Soluble human insulin was infused intravenously at a fixed rate according to estimated body surface area (60 mU $m^{-2}$ $min^{-1}$), and 20% dextrose was infused at a variable rate to achieve target blood glucose concentrations. Blood was drawn from a vein in the non-dominant hand which had been placed within a hot blanket to arterialize venous blood. Samples were taken every 3 to 5 minutes for measurement of whole blood glucose using a Yellow Springs 2300 analyser.

Blood glucose concentrations were stabilized at 4.5 mmol/L for 30 minutes, and the face and word stimuli were presented for learning. Blood glucose was then lowered to 2.5 mmol/L (hypoglycaemia condition) or maintained at 4.5 mmol/L (euglycaemia condition) for one hour. During this hour, subjects were given other cognitive tasks to prevent rehearsal of stimuli (these tasks form the basis of the study described in chapter 6). At the end of the experimental hour the blood glucose concentration was raised to, or maintained at, 4.5 mmol/L and the clamp was discontinued. Subjects were given a meal and allowed to rest for one hour to prevent any 'hangover' effect of hypoglycaemia affecting recall results.

After the recovery hour, blood glucose was measured to ensure euglycaemia. Subjects were then tested for recognition of the face and word stimuli, with simultaneous fMRI acquisition.

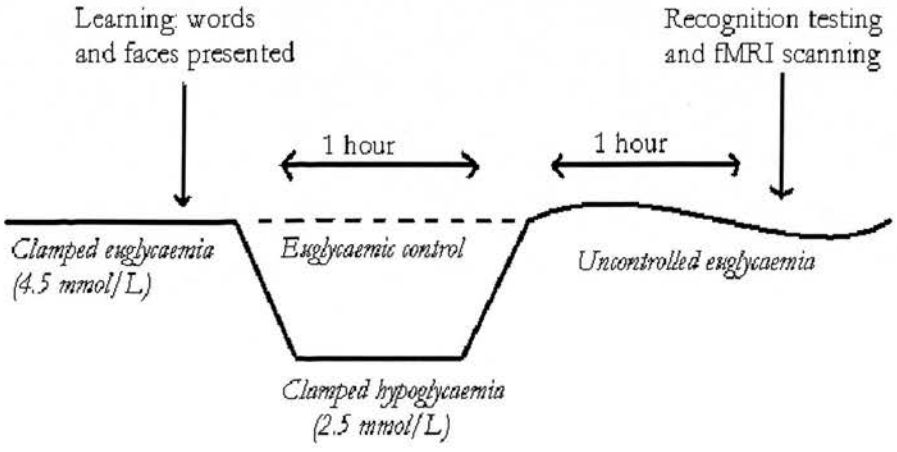



**Figure 4.1.** Sequence of experimental procedures.

### 4.3.4 Memory tests, procedure and analysis

The memory tests were the face and word recognition tests described in section 4.2.2. During the learning phase, the face and word stimuli were presented for variable durations of 3 to 6 seconds on a laptop computer screen at arms length, and subjects were asked to attempt to memorize them. To improve retention, subjects rated each word or face for its attractiveness, although these data were not analysed.

Recognition was tested while subjects were in the MRI scanner. Subjects viewed stimuli on a screen directly above their face, with focal aids if required. Stimuli were presented for variable durations of 3 to 6 seconds using E-prime Version 1.0 (Psychology Software Tools Inc, Pittsburgh, USA) and IFIS 1.09 software (MRI Devices Corporation, Waukesha, USA). Subjects were required to identify targets by pressing a left-hand button, and decoys by pressing a right-hand button.

Corrected memory scores were calculated using the method described in section 4.2.2. Performance was compared for euglycaemia and hypoglycaemia using repeated-measures analysis of variance. Order of glycaemic condition and order of stimulus battery were counterbalanced within the experimental design, and were included as between-subjects factors. Analyses were performed using SPSS version 11.0. It was estimated that with a sample size of 16 and two-tailed $\alpha$ of 0.05, the study had 80% power to detect a difference of 0.75 s.d. and 90% power to detect a difference of 0.88 s.d.

### 4.3.5 Imaging acquisition and analysis

Scanning was carried out at the Brain Imaging Research Centre facilities at the Western General Hospital, Edinburgh. The scanner was a 1.5 Tesla General Electric Signa fitted with Echospeed gradients, using the standard head coil. Acquisition durations were 600 seconds (face paradigm) and 840 seconds (word paradigm). Contiguous gradient echo, echoplanar images (TR 2500 ms, TE 40 ms) were collected from 30 5mm slices

(interleaved acquisition) parallel to the anterior-posterior commissure plane. The matrix acquired was 64 x 64 with an in-plane resolution of 3 x 3 mm. Button responses to the memory tasks were logged simultaneously. A T1-weighted structural scan was acquired after fMRI acquisition. Images in each series were registered to the initial image to correct for head motion. EPI-volumes were then co-registered to the T1-weighted structural volume and aligned to standard coordinates, and data was smoothed with a 6 mm Gaussian kernel to allow for variability in gyral anatomy and location of activation between subjects.

fMRI data were analysed by colleagues at the Centre for Functional Imaging Studies at the University of Edinburgh, using the statistical parametric mapping package SPM99. The fMRI design for the test was event-related. Events were modelled using canonical haemodynamic response functions and six movement regressors. Events were classified by subject responses as 'Correct Hit', 'Correct Rejection, 'Incorrect Hit', or 'Incorrect Rejection'. For a small number of events no response was given, and these were assigned to the relevant incorrect category. First-level fixed-effects T-contrasts were made between the euglycaemia and hypoglycaemia conditions. The T threshold was set at 3.09, and cluster size threshold at 10 voxels.

## 4.4 Results

### 4.4.1 Blood glucose and timing

Blood glucose profiles are depicted in Figure 4.2. For hypoglycaemia studies, the mean blood glucose was 4.36 (SD=0.24) mmol/L during the initial euglycaemic phase, and 2.53 (0.22) mmol/L during the experimental phase. For euglycaemia studies, equivalent means were 4.31 (0.24) mmol/L and 4.42 (0.28) mmol/L respectively.

In hypoglycaemia studies, the mean (SD) time to lower blood glucose from 4.5 mmol/L to a stable level of 2.5 mmol/L was 30 (7) minutes. The interval between the end of the glucose clamp and the start of fMRI scanning was 86 (10) minutes in hypoglycaemia studies, and 85 (15) minutes in euglycaemia studies (P=0.803).

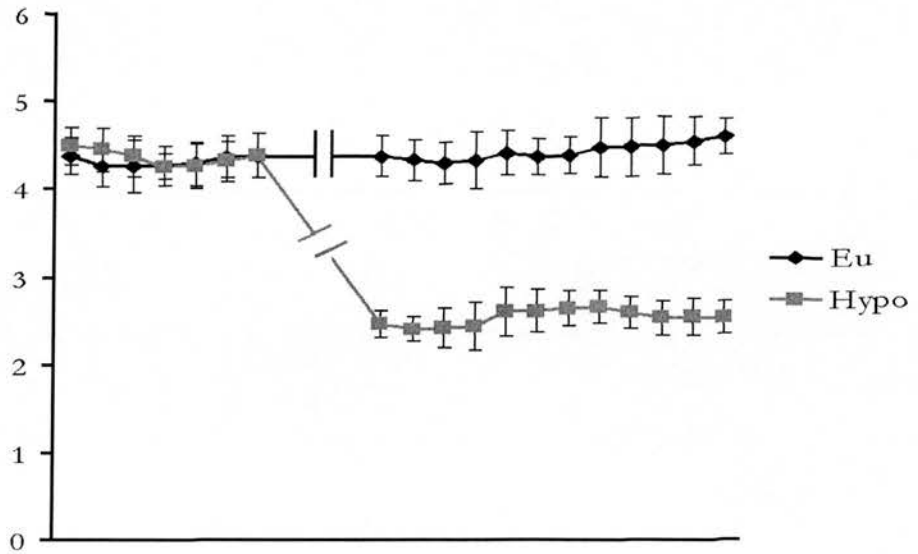



**Figure 4.2.** Mean achieved blood glucose during initial euglycaemic phase and experimental phase (euglycaemia or hypoglycaemia). Plots are broken between phases due to the variable time taken to achieve hypoglycaemia.

### 4.4.2 Memory performance

Memory performance is shown in Table 4.2. Unadjusted data are given for 'Correct Hits', 'Incorrect Hits', 'Correct Rejections' and 'Incorrect Rejections'. 'Adjusted Score', as described in Section 4.2.5, was the principal performance measure. 'Correct Hits'

minus 'Incorrect Hits' (or 'false alarms') is also reported, as this is another commonly used measure of discriminant memory performance.

| Stimulus | Score | Eu | Hypo | P |
|---|---|---|---|---|
| Words | Correct Hits | 39.2 (6.4) | 40.6 (6.7) | 0.367 |
| | Correct Rejections | 69.9 (12.8) | 65.8 (17.1) | 0.113 |
| | Incorrect Hits | 19.8 (12.6) | 20.9 (15.8) | 0.553 |
| | Incorrect Rejections | 10.7 (6.4) | 8.1 (5.5) | 0.030 |
| | CH-IH | 19.4 (12.3) | 19.7 (15.9) | 0.915 |
| | Adjusted Score | 78.0 (8.0) | 77.1 (12.2) | 0.701 |
| Faces | Correct Hits | 24.4 (6.6) | 25.4 (5.7) | 0.624 |
| | Correct Rejections | 36.6 (6.8) | 35.1 (7.2) | 0.335 |
| | Incorrect Hits | 13.3 (6.8) | 14.1 (7.0) | 0.564 |
| | Incorrect Rejections | 15.6 (6.6) | 14.3 (5.5) | 0.524 |
| | CH-IH | 11.1 (7.6) | 11.3 (5.7) | 0.914 |
| | Adjusted Score | 67.2 (8.6) | 66.9 (7.4) | 0.895 |

**Table 4.2.** Mean (SD) performance on memory tasks following euglycaemia (Eu) and hypoglycaemia (Hypo). Performance is reported by six different measures for word and face tasks separately, with P-values for comparison by repeated-measures ANOVA.

Memory performance was almost identical for euglycaemia and hypoglycaemia studies. The adjusted face scores following euglycaemia and hypoglycaemia were 67.2 (SD 8.2) and 66.9 (7.4) respectively ($\eta^2$=0.002, P=0.895). Equivalent adjusted word scores were 78.0 (8.0) and 77.1 (12.2) respectively ($\eta^2$=0.013, P=0.701). For both face and word tasks, adjusted scores were significantly above the chance level of 50 (P<0.001 in both cases).

Although *post hoc* analyses are unreliable, uncorrected scores were examined for evidence of different response strategies. There were no significant differences in unadjusted scores (e.g. 'Correct Hits' and 'Correct Rejections') between hypoglycaemia and euglycaemia. For Incorrect Rejections in the word task, the P-value for the euglycaemia-hypoglycaemia comparison was 0.030, which crosses the standard threshold of 0.05, but with adjustment for multiple comparisons this is clearly non-significant. Overall, subjects gave a 'Hit' response (whether correct or not) for approximately 43% of word items, representing a positive bias (true proportion of targets: 35.7%), and 43%

of face items, representing a slight negative bias (true proportion of targets: 44.4%). The proportion of 'Hit' and 'Reject' responses were not different for euglycaemia and hypoglycaemia conditions.

The study had 90% power to detect a difference of 0.88 standard deviations, and thus it is improbable that there is a true effect of greater magnitude than this. In fact the scores were almost identical for hypoglycaemia and euglycaemia, suggesting that the hypoglycaemic intervention had no effect whatsoever on memory consolidation.

The responses were analysed in detail, looking for evidence that poorly designed test batteries may have contributed to the null result. The mean scores fell appropriately between the floor (50%) and ceiling (100%) limits, suggesting that the tests were neither too hard nor too easy to be sensitive to hypoglycaemic deterioration. Correct response rates were then calculated for individual stimuli to determine their difficulty. This stimulus difficulty showed a normal distribution, with most stimuli being correctly identified on ~70% of occasions, and no very easy or very hard items. (The rationale here is that, had a test battery been composed of half very hard and half very easy items, then following both euglycaemia and hypoglycaemia the former would be answered randomly and the latter correctly. Thus, hypoglycaemia could not affect performance due to a combination of floor and ceiling effects, but the total score would be mid-way between 50% and 100%, giving the appearance of freedom from such effects. There was no evidence of such a problem).

### 4.4.3 Brain activation

Statistical parametric mapping (SPM) is the process by which fMRI data are analysed to identify regions of the brain that show significantly different activation for different experimental conditions (hypoglycaemia *vs* euglycaemia in this case). Voxels ('volume cells') are the smallest volume for which activation can be measured, i.e. the resolution of the scanning process, 3x3x3 mm in this case. Because an exceptionally large number of voxels are imaged within the brain, type 1 statistical error is certain without appropriate correction. If a P-threshold of 0.05 were set for each voxel, the comparison of euglycaemia *vs* hypoglycaemia would pass this threshold due to random chance alone

in thousands of voxels. Such false positives are filtered out in two ways. Firstly, single voxels are ignored; only clusters of contiguous voxels achieving significance are considered. Secondly, P-values for statistical significance are adjusted for multiple comparisons.

SPM outputs are presented in Figures 4.4 – 4.12. Fig 4.3 is a schematic output that illustrates the layout of Figures 4.4 – 4.12. In each case, the output displays the position and size of clusters of at least 10 adjacent voxels that achieve basic statistical significance. The locations of clusters are given as X Y Z coordinates in the table, and depicted in each of the 'glass brain' diagrams. Statistical significance is calculated at both voxel and cluster level, and reported uncorrected and corrected for the entire brain volume. These are fixed-effects analyses, which would normally be preliminary analyses.

Figures 4.4 and 4.5 show comparisons for hypoglycaemia vs euglycaemia for all face stimuli correctly identified, whether as hits or rejections. A cluster low in the right occipital cortex, adjacent to the cerebellum, shows reduced activation after hypoglycaemia (P=0.046). Figures 4.6 and 4.7 show comparisons for all face stimuli correctly identified as hits. The same right occipital cortex cluster shows reduced activation after hypoglycaemia (P=0.016). A cluster in the left frontal cortex tends to increased activation after hypoglycaemia, but does not achieve significance (P=0.08).

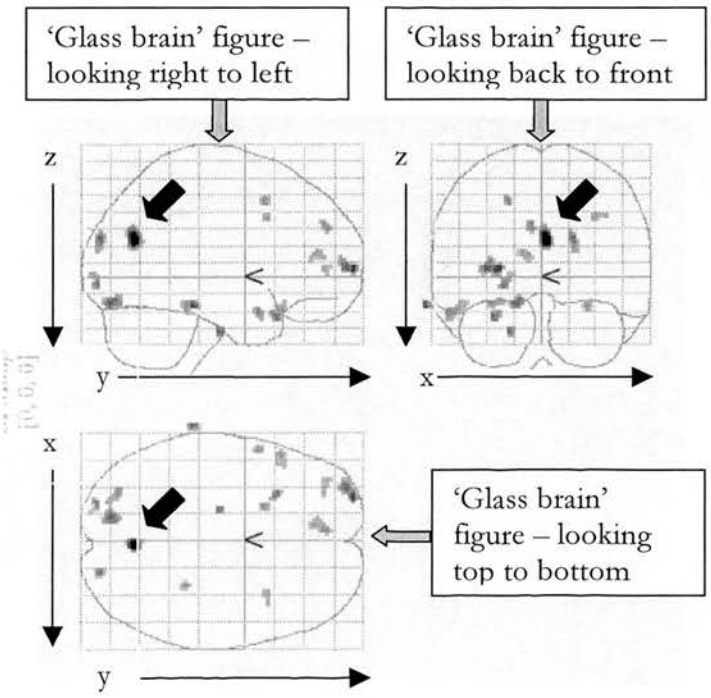
Figure 4.8 shows comparisons for hypoglycaemia vs euglycaemia for all face stimuli, irrespective of response. A cluster in the midline, close to the calcarine sulcus, shows increased activation after hypoglycaemia (P=0.027)

Comparisons for hypoglycaemia vs euglycaemia for word stimuli are shown in Figures 4.9 and 4.10 (all word stimuli correctly identified as hits or rejections) and Figures 4.11 and 4.12 (all word stimuli correctly identified as hits). There are no significant differences.

Thus, significant differences for hypoglycaemia vs euglycaemia were seen in two areas: low in the right occipital cortex, and in the posterior midline. Neither area is known to be associated with memory. Fixed-effects analyses exclude inter-individual variation,

and so do not permit inferences to be made about the effects of hypoglycaemia in the population. The statistical significance levels are borderline using the liberal fixed-effects model, and in the absence of any behavioural (memory performance) results requiring functional correlation, a more conservative random-effects analysis was not performed [26]. It was concluded that the fMRI data do not show evidence of altered brain activation during the recognition memory task following hypoglycaemia.

## Figure 4.3 Statistical Parametric Mapping
## Illustration/Explanation of Subsequent Figures




Each cluster appears in each of the 'glass brain' figures to the left. As an example, the black arrow identifies the same cluster in each figure.

Coordinates for the centre of each cluster are given in the table below. They are relative to 0,0,0 which is

**Statistics:** ***volume summary (p-values corrected for entire volume)***

| set-level | | cluster-level | | | voxel-level | | | | x,y,z {mm} |
|---|---|---|---|---|---|---|---|---|---|
| $p$ | $c$ | $p_{corrected}$ | $k_E$ | $p_{uncorrected}$ | $p_{corrected}$ | $T$ | $(Z_=)$ | $p_{uncorrected}$ | |
| 0.013 | 17 | 0.129 | 74 | 0.003 | 0.038 | 5.06 | ( 5.05) | 0.000 | 2 -68 24 |
| | | 0.304 | 56 | 0.009 | 0.745 | 4.20 | ( 4.19) | 0.000 | -26 66 6 |
| | | | | | 0.885 | 4.07 | ( 4.07) | 0.000 | -34 62 6 |
| | | 0.607 | 40 | 0.023 | 0.921 | 4.03 | ( 4.02) | 0.000 | -16 -82 -16 |
| | | 0.999 | 12 | 0.185 | 0.922 | 4.03 | ( 4.02) | 0.000 | -72 -32 -18 |
| | | 0.924 | 25 | 0.064 | 0.982 | 3.89 | ( 3.89) | 0.000 | 20 -88 24 |
| | | 0.995 | 16 | 0.129 | 0.993 | 3.83 | ( 3.82) | 0.000 | -28 18 -24 |
| | | 1.000 | 11 | 0.203 | 0.995 | 3.81 | ( 3.81) | 0.000 | -20 -16 -34 |
| | | 0.998 | 14 | 0.154 | 1.000 | 3.69 | ( 3.69) | 0.000 | -24 -90 -4 |
| | | 0.995 | 16 | 0.129 | 1.000 | 3.61 | ( 3.61) | 0.000 | -32 -76 -16 |
| | | 0.998 | 14 | 0.154 | 1.000 | 3.60 | ( 3.60) | 0.000 | -56 8 -20 |
| | | 0.997 | 15 | 0.141 | 1.000 | 3.56 | ( 3.56) | 0.000 | -6 50 30 |
| | | 1.000 | 11 | 0.203 | 1.000 | 3.56 | ( 3.56) | 0.000 | 28 -36 -18 |
| | | 0.999 | 12 | 0.185 | 1.000 | 3.55 | ( 3.55) | 0.000 | -22 12 48 |
| | | 0.938 | 24 | 0.068 | 1.000 | 3.53 | ( 3.53) | 0.000 | -54 22 -22 |
| | | 0.960 | 22 | 0.080 | 1.000 | 3.38 | ( 3.38) | 0.000 | -8 46 14 |
| | | 1.000 | 11 | 0.203 | 1.000 | 3.37 | ( 3.37) | 0.000 | 32 14 36 |
| | | | | | 1.000 | 3.22 | ( 3.22) | 0.001 | 40 14 38 |
| | | 0.992 | 17 | 0.119 | 1.000 | 3.31 | ( 3.30) | 0.000 | -30 48 4 |

Significance level for each cluster, corrected for multiple comparisons across entire brain volume

(KEY STATISTIC)

Significance level for each cluster, uncorrected

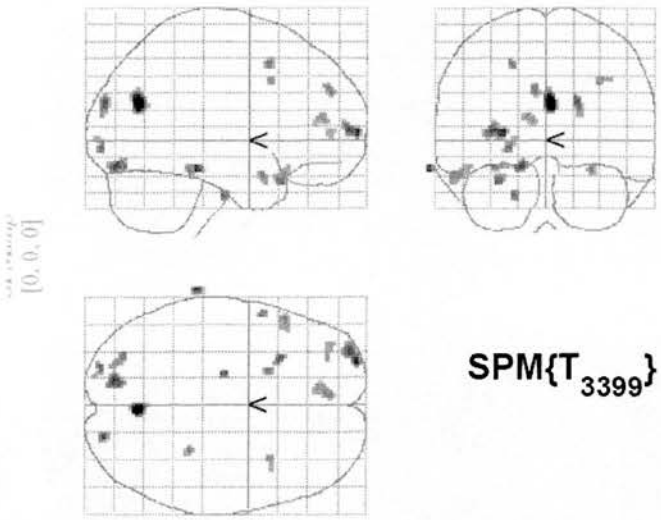
Significance level for single voxels, corrected and uncorrected

Spatial coordinates of cluster centres

## Figure 4.4 Statistical Parametric Mapping

## Faces: Hypoglycaemia Correct > Euglycaemia Correct

('Correct hits' plus 'correct rejections')




COMMENT:

Analysis is restricted to faces correctly identified, either as new or old.

No brain areas show significantly greater activation after hypoglycaemia compared with euglycaemia (cluster-level $P_{corrected}$ >0.05)

**SPMresults:** /mmeyer/Hypo/Analysis/Faces
Height threshold T = 3.09
Extent threshold k = 10 voxels

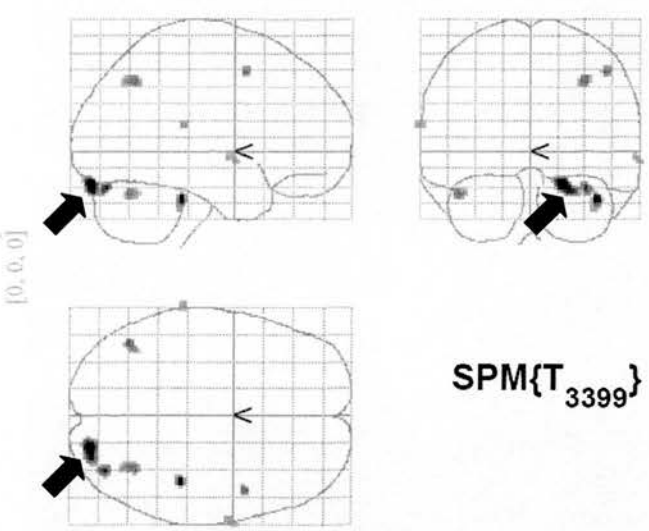
**Statistics:** ***volume summary (p-values corrected for entire volume)***

| set-level | | cluster-level | | | voxel-level | | | | x,y,z {mm} | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $p$ | $c$ | $p_{corrected}$ | $k_E$ | $p_{uncorrected}$ | $p_{corrected}$ | $T$ | ($Z_=$) | $p_{uncorrected}$ | | | |
| 0.012 | 17 | 0.129 | 74 | 0.003 | 0.038 | 5.06 | ( 5.05) | 0.000 | 2 | -68 | 24 |
| | | 0.304 | 56 | 0.009 | 0.745 | 4.20 | ( 4.19) | 0.000 | -26 | 66 | 6 |
| | | | | | 0.885 | 4.07 | ( 4.07) | 0.000 | -34 | 62 | 6 |
| | | 0.607 | 40 | 0.023 | 0.921 | 4.03 | ( 4.02) | 0.000 | -16 | -82 | -16 |
| | | 0.999 | 12 | 0.185 | 0.922 | 4.03 | ( 4.02) | 0.000 | -72 | -32 | -18 |
| | | 0.924 | 25 | 0.064 | 0.982 | 3.89 | ( 3.89) | 0.000 | 20 | -88 | 24 |
| | | 0.995 | 16 | 0.129 | 0.993 | 3.83 | ( 3.82) | 0.000 | -28 | 18 | -24 |
| | | 1.000 | 11 | 0.203 | 0.995 | 3.81 | ( 3.81) | 0.000 | -20 | -16 | -34 |
| | | 0.998 | 14 | 0.154 | 1.000 | 3.69 | ( 3.69) | 0.000 | -24 | -90 | -4 |
| | | 0.995 | 16 | 0.129 | 1.000 | 3.61 | ( 3.61) | 0.000 | -32 | -76 | -16 |
| | | 0.998 | 14 | 0.154 | 1.000 | 3.60 | ( 3.60) | 0.000 | -56 | 8 | -20 |
| | | 0.997 | 15 | 0.141 | 1.000 | 3.56 | ( 3.56) | 0.000 | -6 | 50 | 30 |
| | | 1.000 | 11 | 0.203 | 1.000 | 3.56 | ( 3.56) | 0.000 | 28 | -36 | -18 |
| | | 0.999 | 12 | 0.185 | 1.000 | 3.55 | ( 3.55) | 0.000 | -22 | 12 | 48 |
| | | 0.938 | 24 | 0.068 | 1.000 | 3.53 | ( 3.53) | 0.000 | -54 | 22 | -22 |
| | | 0.960 | 22 | 0.080 | 1.000 | 3.38 | ( 3.38) | 0.000 | -8 | 46 | 14 |
| | | 1.000 | 11 | 0.203 | 1.000 | 3.37 | ( 3.37) | 0.000 | 32 | 14 | 36 |
| | | | | | 1.000 | 3.22 | ( 3.22) | 0.001 | 40 | 14 | 38 |
| | | 0.992 | 17 | 0.119 | 1.000 | 3.31 | ( 3.30) | 0.000 | -30 | 48 | 4 |

## Figure 4.5 Statistical Parametric Mapping

## Faces: Hypoglycaemia Correct < Euglycaemia Correct

('Correct hits' plus 'correct rejections')




COMMENT:

Analysis is restricted to faces correctly identified, either as new or old.

One brain area in the right occipital region shows significantly reduced activation after hypoglycaemia compared with euglycaemia (cluster-level $P_{corrected}$ 0.046). This is centred on 20 -88 -20, and is identified by the black arrow in the figures to the left.

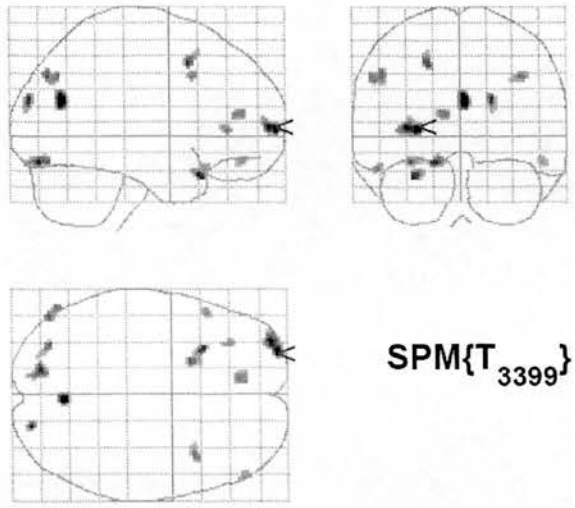
**SPMresults:** /mmeyer/Hypo/Analysis/Faces
Height threshold T = 3.09
Extent threshold k = 10 voxels

**Statistics:** *volume summary (p-values corrected for entire volume)*

| set-level | | cluster-level | | | voxel-level | | | | x,y,z {mm} |
|---|---|---|---|---|---|---|---|---|---|
| $p$ | $c$ | $p_{corrected}$ | $k_E$ | $p_{uncorrected}$ | $p_{corrected}$ | $T$ | ($Z_=$) | $p_{uncorrected}$ | |
| 0.686 | 8 | 0.046 | 96 | 0.001 | 0.050 | 4.99 | ( 4.98) | 0.000 | 20 -88 -20 |
| | | 0.892 | 27 | 0.055 | 0.414 | 4.44 | ( 4.44) | 0.000 | 40 -32 -30 |
| | | 0.924 | 25 | 0.064 | 0.815 | 4.14 | ( 4.14) | 0.000 | 34 -78 -22 |
| | | 0.701 | 36 | 0.030 | 0.993 | 3.83 | ( 3.83) | 0.000 | 32 -62 44 |
| | | 0.909 | 26 | 0.059 | 0.993 | 3.83 | ( 3.82) | 0.000 | -44 -64 -26 |
| | | 1.000 | 11 | 0.203 | 0.997 | 3.77 | ( 3.77) | 0.000 | 46 6 50 |
| | | 1.000 | 11 | 0.203 | 1.000 | 3.36 | ( 3.35) | 0.000 | -68 -32 16 |
| | | 1.000 | 10 | 0.224 | 1.000 | 3.35 | ( 3.35) | 0.000 | 64 -2 -4 |

Figure 4.6 Statistical Parametric Mapping

Faces: Hypoglycaemia Correct Hits > Euglycaemia Correct Hits




COMMENT:

Analysis is restricted to faces correctly identified as old.

No brain areas show significantly greater activation after hypoglycaemia compared with euglycaemia (cluster-level $P_{corrected}$ >0.05)

**SPMresults:** /mmeyer/Hypo/Analysis/Faces
Height threshold T = 3.09
Extent threshold k = 10 voxels

**Statistics:** *volume summary (p-values corrected for entire volume)*

| set-level | | cluster-level | | | voxel-level | | | | x,y,z {mm} |
|---|---|---|---|---|---|---|---|---|---|
| $p$ | $c$ | $p_{corrected}$ | $k_E$ | $p_{uncorrected}$ | $p_{corrected}$ | $T$ | ($Z_=$) | $p_{uncorrected}$ | |
| 0.131 | 13 | 0.277 | 58 | 0.008 | 0.124 | 4.78 | ( 4.77) | 0.000 | 4 -68 22 |
| | | 0.080 | 84 | 0.002 | 0.346 | 4.50 | ( 4.49) | 0.000 | -26 66 6 |
| | | | | | 0.666 | 4.26 | ( 4.25) | 0.000 | -34 62 6 |
| | | 0.540 | 43 | 0.019 | 0.886 | 4.07 | ( 4.07) | 0.000 | -16 -82 -16 |
| | | 0.969 | 21 | 0.086 | 0.924 | 4.02 | ( 4.02) | 0.000 | -30 20 -26 |
| | | 0.950 | 23 | 0.074 | 0.946 | 3.99 | ( 3.98) | 0.000 | 20 -88 24 |
| | | 0.924 | 25 | 0.064 | 0.980 | 3.90 | ( 3.89) | 0.000 | -20 12 46 |
| | | 0.748 | 34 | 0.034 | 0.996 | 3.80 | ( 3.79) | 0.000 | -50 -76 40 |
| | | 0.992 | 17 | 0.119 | 0.998 | 3.77 | ( 3.76) | 0.000 | -32 -76 -16 |
| | | 0.977 | 20 | 0.093 | 1.000 | 3.65 | ( 3.65) | 0.000 | 40 14 38 |
| | | 0.793 | 32 | 0.039 | 1.000 | 3.63 | ( 3.63) | 0.000 | -10 42 14 |
| | | 1.000 | 11 | 0.203 | 1.000 | 3.58 | ( 3.57) | 0.000 | -32 36 4 |
| | | 1.000 | 11 | 0.203 | 1.000 | 3.48 | ( 3.48) | 0.000 | -52 22 -20 |
| | | 1.000 | 10 | 0.224 | 1.000 | 3.37 | ( 3.36) | 0.000 | 52 44 -16 |

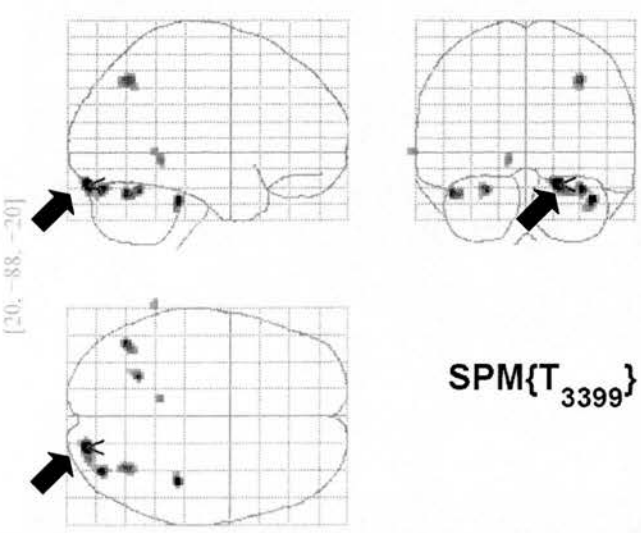
*table shows at most local maxima > 8.0mm apart per cluster*

Height threshold: T = 3.09, p = 0.001 (1.000 corrected)
Extent threshold: k = 10 voxels, p = 0.224 (1.000 corrected)
Expected voxels per cluster, <k> = 7.262
Expected number of clusters, <c> = 9.09

Degrees of freedom = [1.0, 3399.1]
Smoothness FWHM = 7.8 7.8 8.0 {mm} = 3.9 3.9 4.0 {voxels}
Search volume: S = 2205272 mm^3 = 275659 voxels = 4226.2 resels
Voxel size: [2.0, 2.0, 2.0] mm (1 resel = 61.41 voxels)

Figure 4.7 Statistical Parametric Mapping

Faces: Hypoglycaemia Correct Hits < Euglycaemia Correct Hits




COMMENT:

Analysis is restricted to faces correctly identified as old.

One brain area in the right occipital region shows significantly reduced activation after hypoglycaemia compared with euglycaemia (cluster-level $P_{corrected}$ 0.016). This is centred on 20 -88 -20, and is identified by the black arrow in the figures to the left.

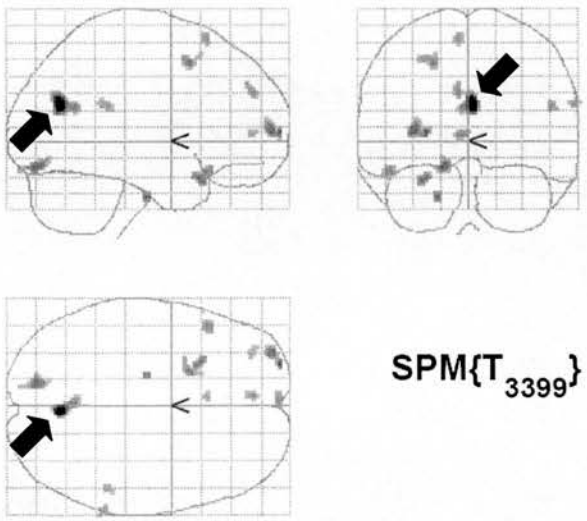
**SPMresults:** /mmeyer/Hypo/Analysis/Faces
Height threshold T = 3.09
Extent threshold k = 10 voxels

**Statistics:** *volume summary (p-values corrected for entire volume)*

| set-level | | cluster-level | | | voxel-level | | | | x,y,z {mm} |
|---|---|---|---|---|---|---|---|---|---|
| $p$ | $c$ | $p_{corrected}$ | $k_E$ | $p_{uncorrected}$ | $p_{corrected}$ | $T$ | $(Z_=)$ | $p_{uncorrected}$ | |
| 0.801 | 7 | 0.016 | 120 | 0.000 | 0.072 | 4.91 | ( 4.90) | 0.000 | 20 -88 -20 |
| | | | | | 0.587 | 4.32 | ( 4.31) | 0.000 | 34 -78 -22 |
| | | 0.793 | 32 | 0.039 | 0.299 | 4.55 | ( 4.54) | 0.000 | 40 -32 -30 |
| | | 0.607 | 40 | 0.023 | 0.841 | 4.12 | ( 4.11) | 0.000 | -46 -64 -26 |
| | | 0.892 | 27 | 0.055 | 0.910 | 4.04 | ( 4.04) | 0.000 | -24 -56 -24 |
| | | 0.382 | 51 | 0.012 | 0.947 | 3.99 | ( 3.98) | 0.000 | 32 -62 44 |
| | | 0.999 | 12 | 0.185 | 1.000 | 3.62 | ( 3.62) | 0.000 | -10 -42 -4 |
| | | 1.000 | 10 | 0.224 | 1.000 | 3.43 | ( 3.43) | 0.000 | -70 -46 0 |

## Figure 4.8 Statistical Parametric Mapping
## Faces: Hypoglycaemia > Euglycaemia

(Irrespective of response or response accuracy)




COMMENT:

Analysis is restricted to all faces irrespective of response.

One brain area in the posterior midline shows significantly increased activation after hypoglycaemia compared with euglycaemia (cluster-level $P_{corrected}$ 0.027). This is centred on 4 -68 22, and is identified by the black arrow in the figures to the left.

**SPMresults:** /mmeyer/Hypo/Analysis/Faces
Height threshold T = 3.09
Extent threshold k = 10 voxels

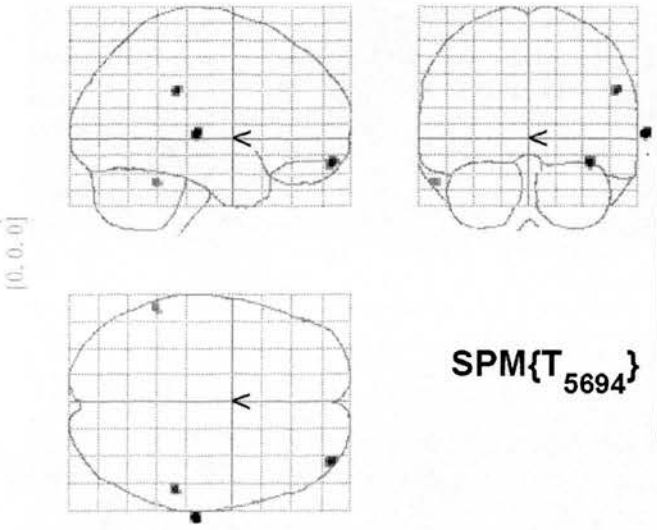
**Statistics:** ***volume summary (p-values corrected for entire volume)***

| set-level | | cluster-level | | | voxel-level | | | | x,y,z {mm} |
|---|---|---|---|---|---|---|---|---|---|
| $p$ | $c$ | $p_{corrected}$ | $k_E$ | $p_{uncorrected}$ | $p_{corrected}$ | $T$ | $(Z_=)$ | $p_{uncorrected}$ | |
| 0.131 | 13 | 0.027 | 108 | 0.001 | 0.023 | 5.16 | ( 5.15) | 0.000 | 4 -68 22 |
| | | | | | 0.999 | 3.70 | ( 3.69) | 0.000 | -2 -60 22 |
| | | 0.456 | 47 | 0.015 | 0.920 | 4.03 | ( 4.02) | 0.000 | -26 66 6 |
| | | | | | 0.947 | 3.98 | ( 3.98) | 0.000 | -34 62 6 |
| | | 0.540 | 43 | 0.019 | 0.956 | 3.97 | ( 3.96) | 0.000 | -16 -82 -16 |
| | | | | | 1.000 | 3.22 | ( 3.21) | 0.001 | -12 -92 -18 |
| | | 0.938 | 24 | 0.068 | 0.979 | 3.91 | ( 3.90) | 0.000 | -28 18 -24 |
| | | 1.000 | 11 | 0.203 | 0.991 | 3.84 | ( 3.84) | 0.000 | -20 -14 -34 |
| | | 0.793 | 32 | 0.039 | 0.999 | 3.71 | ( 3.71) | 0.000 | -22 12 48 |
| | | 0.992 | 17 | 0.119 | 1.000 | 3.67 | ( 3.67) | 0.000 | -4 66 4 |
| | | 0.960 | 22 | 0.080 | 1.000 | 3.62 | ( 3.61) | 0.000 | -48 22 -18 |
| | | 0.998 | 14 | 0.154 | 1.000 | 3.58 | ( 3.58) | 0.000 | -6 50 30 |
| | | 0.999 | 12 | 0.185 | 1.000 | 3.48 | ( 3.48) | 0.000 | -6 22 64 |
| | | 0.999 | 12 | 0.185 | 1.000 | 3.47 | ( 3.47) | 0.000 | 52 -38 22 |
| | | 1.000 | 11 | 0.203 | 1.000 | 3.39 | ( 3.38) | 0.000 | 64 -40 24 |
| | | 1.000 | 10 | 0.224 | 1.000 | 3.31 | ( 3.31) | 0.000 | -30 48 4 |

Figure 4.9 Statistical Parametric Mapping

Words: Hypoglycaemia Correct > Euglycaemia Correct

('Correct hits' plus 'correct rejections')




COMMENT:

Analysis is restricted to words correctly identified, either as old or new.

No brain areas show significantly greater activation after hypoglycaemia compared with euglycaemia (cluster-level $P_{corrected}$ >0.05)

SPMresults: /mmeyer/Hypo/Analysis/Words
Height threshold T = 3.09
Extent threshold k = 10 voxels

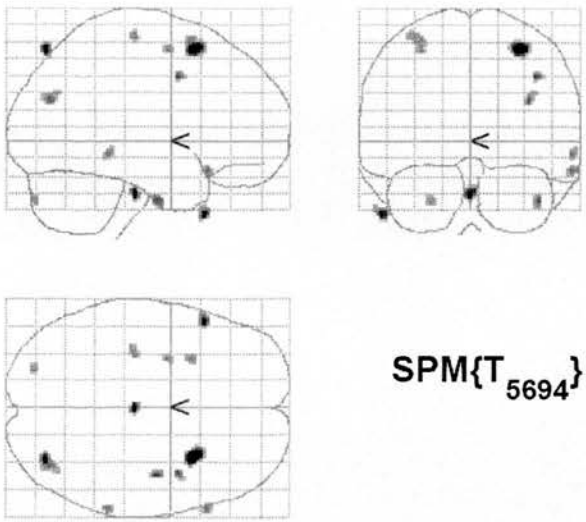
**Statistics:** ***volume summary (p-values corrected for entire volume)***

| set-level | | cluster-level | | | voxel-level | | | | x,y,z {mm} |
|---|---|---|---|---|---|---|---|---|---|
| $p$ | $c$ | $p_{corrected}$ | $k_E$ | $p_{uncorrected}$ | $p_{corrected}$ | $T$ | ($Z_=$) | $p_{uncorrected}$ | |
| 0.980 | 4 | 0.969 | 22 | 0.094 | 0.672 | 4.22 | ( 4.22) | 0.000 | 72 -22 4 |
| | | 0.950 | 24 | 0.082 | 0.998 | 3.73 | ( 3.73) | 0.000 | 38 60 -14 |
| | | 0.996 | 16 | 0.148 | 1.000 | 3.65 | ( 3.65) | 0.000 | 54 -34 30 |
| | | 1.000 | 11 | 0.225 | 1.000 | 3.29 | ( 3.29) | 0.001 | -58 -48 -26 |

## Figure 4.10 Statistical Parametric Mapping

### Words: Hypoglycaemia Correct < Euglycaemia Correct

('Correct hits' plus 'correct rejections'




COMMENT:

Analysis is restricted to words correctly identified, either as old or new.

No brain areas show significantly reduced activation after hypoglycaemia compared with euglycaemia (cluster-level $P_{corrected}$ >0.05)

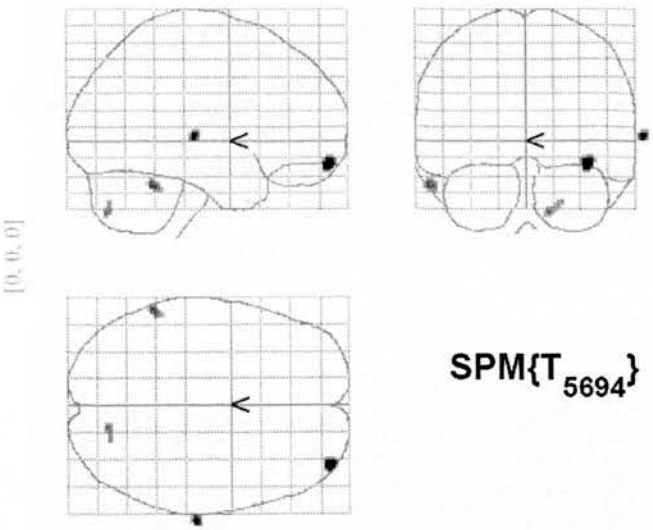
SPMresults: /mmeyer/Hypo/Analysis/Words
Height threshold T = 3.09
Extent threshold k = 10 voxels

**Statistics:** ***volume summary (p-values corrected for entire volume)***

| set-level | | cluster-level | | | voxel-level | | | | x,y,z {mm} |
|---|---|---|---|---|---|---|---|---|---|
| $p$ | $c$ | $p_{corrected}$ | $k_E$ | $p_{uncorrected}$ | $p_{corrected}$ | $T$ | ($Z_=$) | $p_{uncorrected}$ | |
| 0.131 | 13 | 0.128 | 90 | 0.004 | 0.444 | 4.39 | ( 4.39) | 0.000 | 30 12 56 |
| | | 0.829 | 32 | 0.048 | 0.676 | 4.22 | ( 4.22) | 0.000 | 32 -76 58 |
| | | 0.976 | 21 | 0.101 | 0.805 | 4.12 | ( 4.12) | 0.000 | 0 -22 -32 |
| | | 0.950 | 24 | 0.082 | 0.949 | 3.95 | ( 3.95) | 0.000 | -54 20 -44 |
| | | 1.000 | 10 | 0.247 | 0.999 | 3.70 | ( 3.69) | 0.000 | 40 4 40 |
| | | 0.982 | 20 | 0.109 | 1.000 | 3.66 | ( 3.66) | 0.000 | 42 -8 -36 |
| | | 0.899 | 28 | 0.062 | 1.000 | 3.65 | ( 3.65) | 0.000 | 36 -74 26 |
| | | 0.998 | 14 | 0.174 | 1.000 | 3.60 | ( 3.60) | 0.000 | 62 22 -18 |
| | | 1.000 | 11 | 0.225 | 1.000 | 3.48 | ( 3.48) | 0.000 | 62 -38 -8 |
| | | 1.000 | 10 | 0.247 | 1.000 | 3.46 | ( 3.46) | 0.000 | -30 -2 56 |
| | | 0.982 | 20 | 0.109 | 1.000 | 3.45 | ( 3.44) | 0.000 | -30 12 62 |
| | | 0.990 | 18 | 0.126 | 1.000 | 3.43 | ( 3.42) | 0.000 | -34 -22 64 |
| | | 1.000 | 10 | 0.247 | 1.000 | 3.38 | ( 3.38) | 0.000 | -24 -92 -38 |

Figure 4.11 Statistical Parametric Mapping

Words: Hypoglycaemia Correct Hits > Euglycaemia Correct Hits




COMMENT:

Analysis is restricted to words correctly identified as old.

No brain areas show significantly increased activation after hypoglycaemia compared with euglycaemia (cluster-level $P_{corrected}$ >0.05)

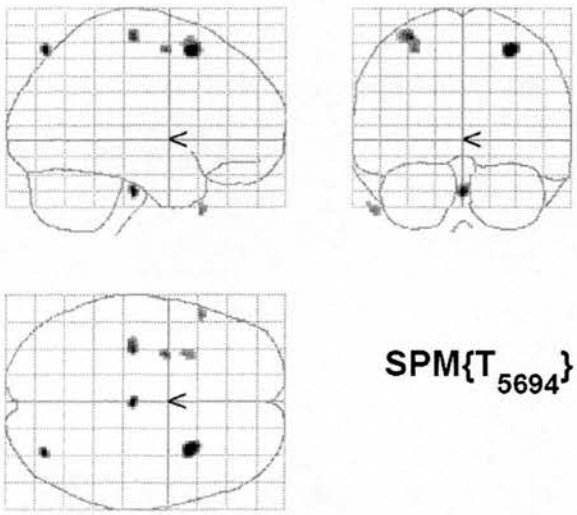
**SPMresults:** /mmeyer/Hypo/Analysis/Words
Height threshold T = 3.09
Extent threshold k = 10 voxels

**Statistics:** ***volume summary (p-values corrected for entire volume)***

| set-level | | cluster-level | | | voxel-level | | | | x,y,z {mm} |
|---|---|---|---|---|---|---|---|---|---|
| $p$ | $c$ | $p_{corrected}$ | $k_E$ | $p_{uncorrected}$ | $p_{corrected}$ | $T$ | ($Z_=$) | $p_{uncorrected}$ | |
| 0.980 | 4 | 0.578 | 44 | 0.023 | 0.664 | 4.23 | ( 4.23) | 0.000 | 38 60 -14 |
| | | 0.990 | 18 | 0.126 | 0.936 | 3.97 | ( 3.97) | 0.000 | 72 -22 4 |
| | | 0.960 | 23 | 0.088 | 1.000 | 3.60 | ( 3.60) | 0.000 | -60 -48 -28 |
| | | 0.993 | 17 | 0.137 | 1.000 | 3.45 | ( 3.45) | 0.000 | 14 -76 -44 |
| | | | | | 1.000 | 3.25 | ( 3.25) | 0.001 | 22 -74 -38 |

Figure 4.12 Statistical Parametric Mapping

Words: Hypoglycaemia Correct Hits < Euglycaemia Correct Hits




COMMENT:

Analysis is restricted to words correctly identified as old.

No brain areas show significantly reduced activation after hypoglycaemia compared with euglycaemia (cluster-level $P_{corrected}$ >0.05)

**SPMresults:** /mmeyer/Hypo/Analysis/Words
Height threshold T = 3.09
Extent threshold k = 10 voxels

**Statistics:** ***volume summary (p-values corrected for entire volume)***

| set-level | | cluster-level | | | voxel-level | | | | x,y,z {mm} |
|---|---|---|---|---|---|---|---|---|---|
| $p$ | $c$ | $p_{corrected}$ | $k_E$ | $p_{uncorrected}$ | $p_{corrected}$ | $T$ | ($Z_=$) | $p_{uncorrected}$ | |
| 0.802 | 7 | 0.146 | 77 | 0.004 | 0.273 | 4.55 | ( 4.54) | 0.000 | 30 14 56 |
| | | 0.960 | 23 | 0.088 | 0.861 | 4.07 | ( 4.07) | 0.000 | 0 -22 -32 |
| | | 0.987 | 19 | 0.117 | 0.912 | 4.01 | ( 4.01) | 0.000 | 32 -76 56 |
| | | 0.620 | 42 | 0.026 | 0.987 | 3.84 | ( 3.84) | 0.000 | -32 -22 64 |
| | | 0.999 | 12 | 0.206 | 1.000 | 3.66 | ( 3.65) | 0.000 | -30 -2 56 |
| | | 1.000 | 11 | 0.225 | 1.000 | 3.52 | ( 3.52) | 0.000 | -54 20 -44 |
| | | 0.899 | 28 | 0.062 | 1.000 | 3.46 | ( 3.46) | 0.000 | -30 12 60 |

## 4.5 Discussion

### 4.5.1 Discussion – results and interpretation of present study

In the present study, one hour of hypoglycaemia at 2.5 mmol/L occurring between the learning and recognition phases of a memory test did not affect memory performance. It is possible that hypoglycaemia has no effect on consolidation, but at least five alternative explanations can be proposed. In this sense, the null findings are helpful to further research.

First, the study may have been underpowered to detect an effect of hypoglycaemia on memory. Advance sample size estimates were limited by a lack of previous data. A sample size of 16 was selected because previous hypoglycaemia studies that have yielded positive results have generally been of a similar or smaller size. There are significant additional logistic and financial issues associated with fMRI studies, which would have made it difficult to complete a larger study; 16 subjects is quite a large number for an fMRI study. Our advance power calculation indicated that the study should have good power to detect a difference of 0.75-0.88 standard deviations on any measure. This is a 'large' effect, according to Cohen's conventions [27]; if the effects of hypoglycaemia were more modest, then the study would be underpowered. However, although not a statistically rigorous observation, it is notable that performance in the present study was almost identical following euglycaemia and hypoglycaemia, which does not support the possibility of an effect failing to achieve significance.

Second, the consolidation process may have been complete by the time hypoglycaemia was achieved (20-40 minutes after stimulus exposure). Unfortunately, despite extensive research, there is no clear account of the duration of the consolidation process. Diverse interventions have been shown to affect recall of memories that were acquired minutes or hours beforehand, including drug administration [28,29,30] and traumatic concussion [31]. There is also a very large body of evidence for the improving effects of sleep, immediately or overnight, on memory consolidation [32,33]. These studies suggest that consolidation takes place during the minutes or hours after stimulus exposure. On the

other hand, studies of patients with hippocampal lesions have shown a temporal gradient for amnesia over years [34,35,36], suggesting a chronic transfer of memory from hippocampal to other (perhaps cortical) sites. This has been supported by a recent fMRI study in which recognition-associated brain activation was measured 1, 2, 30 and 90 days after stimulus exposure, and was found to progressively decrease in the hippocampus and increase in the ventral medial prefrontal region [32]. Thus, consolidation appears to be a chronic and heterogeneous process. It is possible that there is a very early phase of consolidation (e.g. less than 20 minutes after learning) which is susceptible to acute, reversible, metabolic derangement. If so then a different result might be expected if hypoglycaemia could be induced instantaneously, but this would not be relevant to the daily life of people with diabetes, in whom hypoglycaemia develops more slowly, and often over hours [37].

Third, the level of hypoglycaemia induced (2.5 mmol/L) may have been insufficient. There is some evidence that different cognitive processes are differentially sensitive to hypoglycaemia [38], and although it is known that the entire memory process from learning to recall is affected by hypoglycaemia at 2.5 mmol/L [17,18,19], more profound hypoglycaemia may be necessary to disrupt consolidation. Anterograde amnesia is well recognised after electro-convulsive therapy, epileptic seizure, general anaesthesia and alcohol intoxication [39,40,41,42,43] in addition to traumatic injury and other pharmaceutical interventions as cited above, suggesting that any cause of major cerebral dysfunction may have an amnesic effect.

Fourth, it is possible that the brain may work harder, or differently by recruitment of other brain regions, to achieve the same result during hypoglycaemia. However, the functional imaging data revealed only two brain areas with apparently different activation for the hypoglycaemia and euglycaemia conditions. These brain regions are not known to be associated with memory, and a liberal fixed-effects analysis yielded only borderline statistical significance. We conclude that the functional imaging showed no significant effect of hypoglycaemia.

Fifth, the present study measured recognition of stimuli, whereas most other studies of memory during hypoglycaemia have measured recall (retrieval of information). Testing

of recognition was necessary for a number of reasons relating to the use of fMRI, but it is possible that less robust memory encoding is sufficient for recognition, and hence that recognition would be less disrupted by hypoglycaemia. However, in other studies, a recognition task has been adequate to show both changes in memory performance and fMRI brain activation following an intervention to affect consolidation [32]. More generally, visual recognition tasks have formed an important part of functional imaging studies of memory [23]. The recognition material (i.e. the particular faces and words) used in the present study was novel, but subjects were clearly able to identify stimuli with above chance accuracy, and there was no evidence of floor or ceiling effects at either the test or the item level (see section 4.3.3), suggesting that the test should have been sensitive to memory dysfunction if this had occurred.

The present study was intended to study a possible consequence of insulin-induced hypoglycaemia, which is a common occurrence for people with insulin-treated diabetes. Ideally, diabetic subjects would have been studied, but to do this would have necessitated continuing the euglycaemic clamp during the MRI scanning to prevent hypo- or hyperglycaemia, which could affect memory performance. Medical infusion devices cannot be taken into MRI rooms because of the powerful magnetic field, and so infusions must be managed remotely from an adjacent room using very long tubing. Such facilities were not available at the site where this study was performed. The use of non-diabetic subjects may limit the applicability of the results to diabetes. However, as discussed in section 2.5.3, recurrent hypoglycaemia in diabetes appears to induce an adaptive process such that cognitive function is relatively preserved during hypoglycaemia in people with diabetes. It therefore seems unlikely that memory impairment would have been seen in the present study had diabetic subjects been studied.

### 4.5.2 Discussion – recent studies of memory consolidation

In a recently-published study, Jauch-Chara *et al* examined the effects of hypoglycaemia on memory consolidation overnight in 16 type 1 diabetic and 16 non-diabetic subjects [44]. Forty noun pairs were learned; during testing, the first word of each pair was presented and the subject required to name the second word. At the start of each study

in the late evening, learning and testing of the same 40 word-pairs was repeated until a minimum of 60% were recalled correctly, after which subjects slept overnight. Shortly after the onset of stage 2 sleep, an insulin infusion was commenced: on experimental nights, a steady fall in plasma glucose was induced over about an hour to a nadir of 2.2 mmol/l (non-arterialized venous measurement). Recall was then retested in the morning. The study was repeated on control nights, when plasma glucose was maintained above 3.86 mmol/l. Overall there was a small but statistically significant improvement overnight across all studies, consistent with the known effects of sleep on consolidation, but the improvement was greater on control nights than on hypoglycaemia nights. The number of word pairs recalled after control nights was about 5% higher than after hypoglycaemia nights. The results suggest that overnight hypoglycaemia disrupted consolidation of memories formed during the evening.

If one discounts issues of statistical power and concludes that the effect of hypoglycaemia was nil in the present study, then why would an effect of hypoglycaemia have been seen in the study by Jauch-Chara *et al*? They studied type 1 diabetic as well as non-diabetic subjects, but as the effect of hypoglycaemia was greatest in non-diabetic subjects this is unlikely to be the explanation. It also seems unlikely that recall of words should be inherently more sensitive to hypoglycaemia than recognition of words. The overall difficulty of the tests was very similar with scores of 75-80% correct in both studies. In the study by Jauch-Chara *et al*, the hypoglycaemic nadir was only slightly lower than in the present study (2.2 vs. 2.5 mmol/L), and total hypoglycaemic exposure was less as the nadir was reached after a slow fall of 60 minutes, compared to an hour at 2.5 mmol/L in the present study. The interval between learning and hypoglycaemia was not reported by Jauch-Chara *et al*, but was probably at least an hour longer than in the present study; it is unlikely that a longer interval would cause greater impairment. The most plausible explanation is that memory consolidation is more susceptible during sleep.

Another recent study of consolidation is worthy of consideration. Takashima *et al* studied 24 healthy volunteers using a very similar memory recognition task to that of the present study [32]. Subjects viewed images of landscapes with and without buildings, before and after a three hour rest period during which sleep was encouraged. About 30

minutes after the second session of stimulus exposure, recognition was tested with simultaneous fMRI scanning (a similar methodology to the present study). There was no significant correlation between duration of REM/stage 1 sleep and memory performance. There was a significant correlation for stage 2 sleep, applying equally to stimuli viewed before and after sleep, suggesting that the sleep may simply have refreshed subjects for the recognition testing. There was a significant correlation for slow-wave (deep) sleep, of greater magnitude (with statistical significance) for stimuli viewed before sleep, suggesting that deep sleep had a positive effect on memory consolidation.

From these two recent studies, suggestions for future research may be derived. First, the need for an appropriate sample size is re-emphasized. Jauch-Chara *et al* did not report a significant effect of hypoglycaemia in their subsets of 16 diabetic and 16 non-diabetic subjects, but in all 32 subjects combined. Second, both studies assessed consolidation during sleep, which appears to be a time of active re-processing of memory [45], and therefore perhaps a time of increased susceptibility to interventions such as hypoglycaemia.

### 4.5.3 Discussion – other functional brain imaging studies during hypoglycaemia

Other researchers have managed to examine functional brain imaging during hypoglycaemia. Rosenthal *et al* used fMRI to examine the effects of hypoglycaemia (2.5 mmol/L) on brain activation during cognitive tasks (finger tapping and reaction time) in healthy subjects [46]. During hypoglycaemia there was increased activation in the parietal association area, and decreased activation in the visual cortex, cerebellum, hippocampus and pre-motor cortex by comparison with euglycaemia. The hippocampus was the only one of these areas that was not activated by the cognitive tasks. Teves *et al* used positron emitting tomography (PET) to measure cerebral blood flow (CBF) during passive euglycaemia and hypoglycaemia (3.0 mmol/L) in healthy subjects [47]. Global CBF fell by 6-8% during hypoglycaemia, with relatively increased CBF in the thalamus, medial prefrontal cortex and right orbital prefrontal cortex, and a 26% relative decrease in CBF in the hippocampus. An earlier Edinburgh study in

diabetic subjects using single photon emission computed tomography (SPECT) reported increased CBF in the frontal cortices and right thalamus during hypoglycaemia, with decreases in the right cingulate and putamen [48].

It is impossible to amalgamate these studies to obtain a coherent account of changes in activation throughout the brain. However, the hippocampus stood out as having reduced fMRI activation or CBF during hypoglycaemia in the studies by Rosenthal *et al* and Teves *et al* [46,47]. Given the sensitivity of the hippocampus to hypoglycaemic damage [8,9,10], it seems plausible that hippocampal dysfunction during moderate hypoglycaemia contributes to memory impairment.

### 4.5.4 Discussion - summary

In summary, one hour of hypoglycaemia at 2.5 mmol/l after stimulus exposure did not affect subsequent recognition performance, and did not alter brain activation patterns during recognition. A large effect of moderate hypoglycaemia on memory consolidation can be excluded, and thus the study provides some limited reassurance relevant to diabetic daily life. In retrospect, the study was underpowered to detect an effect of hypoglycaemia on memory comparable to effects seen in other studies, and future studies should probably aim to recruit 30 or more subjects. It is possible that inducing hypoglycaemia during sleep, varying the degree and duration of hypoglycaemia, or the interval between stimulus exposure and testing, would yield different results. It is also possible that moderate hypoglycaemia could disrupt performance on other memory tasks (e.g. recall rather than recognition) which may better represent the role of memory in everyday life; this is the hypothesis of the next chapter.

## Acknowledgements

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# Chapter 5

# Effects of Hypoglycaemia on Learning, Recall and Prospective Memory

## 5.1 Introduction

In all previous studies of the effects of hypoglycaemia on memory function (with the exception of the study described in Chapter 4), both the acquisition (learning) and recall of material took place during hypoglycaemia. Deterioration in memory performance during hypoglycaemia may have resulted from impairment of learning, consolidation (transfer of information from short- to long-term memory), recall, or any combination of these. The present study was designed to investigate whether these processes are independently sensitive to hypoglycaemia by separating the acquisition and recall phases of memory tasks so that only one occurred during hypoglycaemia.

Conventional laboratory memory tests tend to involve tasks such as learning lists of words, prose passages, and geometrical shapes. Such tests may not reflect the memory demands of daily living, which require coordination of multiple cognitive processes such as planning and vigilance, and therefore may not have ecological relevance [1,2]. Recall of retrospective events has been distinguished from prospective memory (remembering intended actions, or "remembering to remember"), and tests of prospective memory may have greater ecological validity [3]. Titov and Knight developed a novel test of prospective memory in which subjects memorized a shopping list and then viewed a video of a pedestrian journey around an urban shopping area, reporting items to be purchased when specified shops appeared [4]. This video-based memory task had good test-retest reliability. It also had good criterion validity: it correlated well with performance on the same task when subjects actually walked through the shopping area. Comparative scores were obtained for 35 subjects, yielding a correlation of $r=0.71$, $P<0.001$, and no significant effect of study type (video vs. *in vivo*) in ANOVA. A similar

video task was created for the present study to explore the effects of hypoglycaemia on prospective memory in a practical setting.

Evidence has accumulated to show that recurrent exposure to hypoglycaemia in people with type 1 diabetes results in cerebral adaptation, such that cognitive performance is relatively preserved during hypoglycaemia [5,6,7,8,9,10]. Repeated exposure to hypoglycaemia can also result in impaired hypoglycaemia awareness, and a shared mechanism for the two phenomena has been proposed [11]. Thus, in studies of people with type 1 diabetes, it is important that subjects with impaired hypoglycaemia awareness (which may be a surrogate marker of cerebral adaptation to hypoglycaemia) are considered separately from those with normal hypoglycaemia awareness.

The aims of the present study were: (1) to assess the effects of acute hypoglycaemia on different memory processes (acquisition and recall) for conventional memory tasks; (2) to assess the effects of acute hypoglycaemia on a novel prospective memory task; (3) to compare these effects in people with type 1 diabetes who had either normal or impaired hypoglycaemia awareness.

## 5.2 Methods

The study protocol was approved by the Lothian medical research ethics committee, and all subjects gave informed consent for participation.

### 5.2.1 Subjects

Subjects were required to have a diagnosis of type 1 diabetes, age between 18-40 years, a body mass index (BMI) between 20-30 kg $m^{-2}$, and $HbA1_c$ values between 7 and 10%. They were ineligible if they had any significant current medical condition or contra-indication to experimental hypoglycaemia. Female patients were eligible only if a pregnancy test was negative.

Potential subjects were asked to grade their hypoglycaemia awareness on a scale from 1 to 7 [12], and hypoglycaemia history was also discussed. People who chose 1-2 and reported no history of severe hypoglycaemia or significant change in their warning symptoms were categorized as having normal hypoglycaemia awareness (NHA). People who chose 3-7 and reported diminution of hypoglycaemic symptoms and episodes of unrecognised hypoglycaemia were categorized as having impaired hypoglycaemia awareness (IHA) [12]. People whose self-rated awareness appeared inconsistent with their hypoglycaemia history were ineligible, on the grounds that their awareness status was uncertain.

In total, 36 subjects were recruited (20 NHA, 16 IHA); characteristics are given in table 5.1. Microvascular complications were defined as any clinical diagnosis of diabetic retinopathy, neuropathy or nephropathy, the latter also requiring urine albumin:creatinine ratio persistently above the local reference maximum or serum creatinine > 150 μmol/l. The IHA group had significantly longer duration of diabetes ($t=3.937$, $df=34$, $P<0.001$) and more microvascular complications ($\chi^2 = 5.994$, $df=1$, $P=0.013$). Other comparisons were non-significant.

| | Normal awareness | Impaired awareness |
|---|---|---|
| Number | 20 | 16 |
| Male:female | 12 : 8 | 6 : 10 |
| Median (range) age (years) | 29 (19-44) | 33.5 (22-43) |
| Median (range) diabetes duration (years) | 3.8 (1.1-20) | 15.5 (2-35) |
| $HbA1_c$ (%) | 7.8 (1.3) | 8.4 (1.8) |
| BMI (kg $m^{-2}$) | 25.8 (2.2) | 26.8 (3.6) |
| Number (%) with microvascular complications | 1 (5) | 6 (38) |

**Table 5.1.** Subject characteristics. Data are mean (SD) unless stated otherwise. Non-diabetic reference range for $HbA_{1c}$ 5.0-6.5%.

### 5.2.2 Glucose clamp procedure

Each subject underwent one hypoglycaemic and one euglycaemic glucose clamp, separated by at least two weeks. Subjects were not informed of the order in which these occurred. On the evenings before studies, subjects took their normal insulin, fasted from 22.00h (consuming only water) and omitted insulin on the morning of the study. Subjects were encouraged to avoid hypoglycaemia during the 48 hours before planned studies, by reducing insulin doses if necessary. Studies were postponed if subjects detected any episode of hypoglycaemia by symptoms or routine blood testing during this period, resulting in six postponements (two NHA, four IHA).

An antecubital vein and a dorsal hand vein were cannulated in the non-dominant arm for infusions and blood sampling. The hand was placed within a heated blanket to arterialize venous blood. Arterialized venous samples were drawn every 5 minutes, and whole-blood glucose was measured using a Yellow Springs 2300 analyser. An infusion of soluble human insulin (Actrapid, NovoNordisk) was started at 1.5 mU $kg^{-1}$ $min^{-1}$, and

20% glucose solution was infused at a variable rate to achieve desired blood glucose concentrations.

The blood glucose concentration was initially stabilized at 4.5 mmol/l (euglycaemia), and maintained for 40 minutes. During the hypoglycaemia studies, the blood glucose was then lowered over 20 minutes to 2.5 mmol/l and maintained for 60 minutes, then raised to 4.5 mmol/l and maintained for a further 75 minutes. During the euglycaemia studies, blood glucose was maintained at 4.5 mmol/l throughout. The study outline is depicted in Figure 5.1.

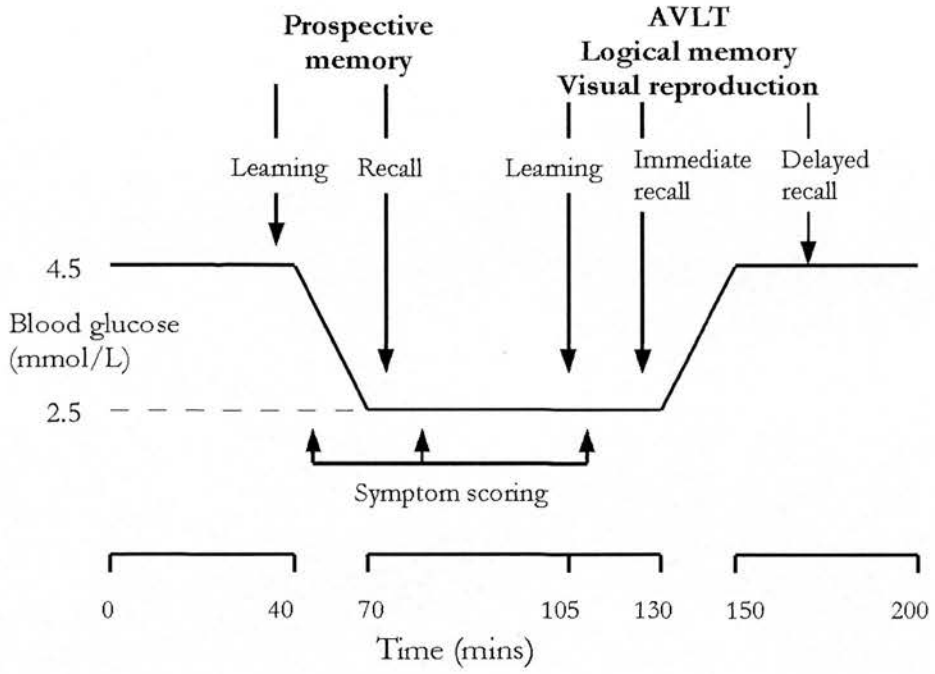


**Figure 5.1.** Study outline. The same timing was adhered to for euglycaemia studies.

### 5.2.3 Memory tests

Two parallel versions of each test were available, which were combined to give two batteries. The order in which subjects were exposed to these batteries and the hypoglycaemia-euglycaemia order were counterbalanced within the NHA and IHA groups.

*Prospective memory.* This was a novel test, based on a method developed by Titov and Knight [4]. Two videos showing the view of a pedestrian journeying around central Edinburgh were created, and for each a 'shopping list' of 21 tasks was written on 21 cards. These were made up of *Buy* tasks (e.g. "Buy tennis balls at A&B Sports"), *Do* tasks (e.g. "Book a table at Smith's restaurant") and *Question* tasks (e.g. "What is the advertised loan rate at the Mercantile Bank?"). Subjects were asked to read the cards twice at a normal reading pace, placing each card face down once read. Subjects then watched the video, and scored points for answering questions or stating actions at the relevant places. These were not brought to subjects' attention, and points were not awarded for answers given at inappropriate times. Pilot studies indicated that the two videos were of equivalent difficulty. Each lasted approximately 10 minutes, and were similar in terms of numbers of shops passed (about 80). In the present study, subjects read the cards (learning) during initial euglycaemia, and recall was tested after approximately 40 minutes during the experimental period of induced hypoglycaemia/euglycaemia. Thus, the experimental manipulation examined the effects of hypoglycaemia on recall.

*Conventional memory tests.* For the following memory tests, acquisition and immediate recall were tested during the experimental hour (euglycaemia or hypoglycaemia). Delayed recall was tested 90 minutes later during euglycaemia, without further exposure to the learned material. Thus, the experimental manipulation here examined the effects of hypoglycaemia on acquisition (learning).

(1) Auditory Verbal Learning Test (AVLT) – immediate and delayed [13]. Fifteen words were read aloud, and the subject asked to say them back immediately. This was repeated with the same words four times in immediate succession, and the immediate score was the sum of correct responses for the five trials (maximum 75). Later, the subject was asked to recall the words, but this time without them being read aloud, giving a delayed score (maximum 15).

(2) Logical Memory Test – immediate and delayed. In this subtest from the Wechsler Memory Scales – Revised [14], a short story was read to the subject, who tried to recount it immediately. Points were obtained for recollection of specific details and story themes.

(3) Visual Reproduction – immediate and delayed, from the Wechsler Memory Scales - Revised [14]. Each of five line drawings was shown to the subject for 10 seconds and then hidden, and the subject tried to draw the design from memory. Reproductions were scored according to strict criteria.

### 5.2.4 General cognitive function tests

At the beginning of the experimental period (i.e. after achievement of stable hypoglycaemia, or at the equivalent time during euglycaemia studies), subjects performed tests of 4-choice reaction time, Trail-Making B and the Digit Symbol Substitution Test as measures of general cognitive processing speed.

### 5.2.5 Symptom scores

Subjects scored their symptoms of hypoglycaemia at baseline and during the experimental period using the Edinburgh hypoglycaemia scale [15].

### 5.2.6 Statistical analysis

Scores on memory tasks were compared using repeated-measures analysis of variance (ANOVA). Memory test score during euglycaemia versus hypoglycaemia was the repeated measure (within-subjects factor). Hypoglycaemia awareness, order of hypoglycaemia-euglycaemia and order of test battery were between-subjects factors. Statistical significance was accepted at $P<0.05$ and $\eta^2$ was used to indicate effect size (proportion of variance explained, comparable to $r^2$ in correlation or linear regression). Analyses were performed using SPSS 11.0.

## 5.3 Results

### 5.3.1 Blood glucose

During the hypoglycaemia condition, mean blood glucose was 2.5 (SD 0.2) mmol/l in the NHA group, and 2.5 (0.2) mmol/l in the IHA group (P=0.468). During euglycaemia, mean blood glucose was 4.5 (0.2) mmol/l (NHA) and 4.5 (0.3) mmol/l (P=0.643) (IHA).

### 5.3.2 Symptoms

Total symptom scores did not change during euglycaemia. During the hypoglycaemia condition mean symptom scores rose in both NHA subjects (baseline 23.2 (4.4) vs. experimental 44.1 (22.2); P<0.001) and IHA subjects (22.9 (7.0) vs. 28.8 (8.3); P=0.001). The increment in symptom scores was significantly greater in the NHA group (interaction between glycaemic condition and awareness status: P=0.002).

### 5.3.3 General cognitive function tasks

In NHA subjects, performance on all tests was poorer during hypoglycaemia compared with euglycaemia (4-choice reaction time and Digit Symbol Substitution – P<0.001; Trail-Making B – P<0.05). In IHA subjects, performance also deteriorated, but this was not statistically significant for any test. The interaction between the experimental condition and the hypoglycaemia awareness state was significant for DSST (P<0.01), confirming a greater effect of hypoglycaemia in NHA subjects than in IHA subjects for this task.

These data are not reported or discussed in detail in this thesis, but are examined in detail elsewhere.

### 5.3.4 Memory tasks

Results are given in Table 5.2. There were no significant effects of order of exposure to glycaemic condition or test battery.

*Comparison of effect of hypoglycaemia in NHA and IHA subjects.* The interaction between glycaemic state and hypoglycaemia awareness designation (hereafter the glycaemia-awareness interaction) was not significant for any tests. This means that there was no significant difference in the effects of hypoglycaemia on cognitive function in the NHA and IHA groups. In some cases, there was a statistically significant effect of hypoglycaemia in one group but not the other; however, in each case hypoglycaemia was associated with poorer performance in both groups, and achievement of statistical significance in one group but not the other may have been due to chance. In the absence of significant glycaemia-awareness interactions, the effect of hypoglycaemia on memory performance was determined for all subjects combined.

*Prospective memory.* Recall was significantly impaired in all patients combined during hypoglycaemia ($\eta^2$=0.255, P=0.004). IHA subjects performed better overall than NHA subjects (P=0.018).

*Immediate verbal memory.* Immediate recall was significantly impaired during hypoglycaemia for AVLT ($\eta^2$=0.268, P=0.003) and logical memory ($\eta^2$=0.233, P=0.007) in all subjects combined. The IHA group performed significantly better than the NHA group on the logical memory task (P=0.030).

*Delayed verbal memory.* Delayed recall was significantly impaired during hypoglycaemia for both AVLT ($\eta^2$=0.373, P<0.001) and logical memory ($\eta^2$=0.315, P<0.001) in all subjects combined. Again, the IHA group performed better overall on the logical memory task (P=0.031). Hypoglycaemia had no effect on the proportion of information retained (delayed score as a percentage of immediate score).

*Visual memory.* No significant effects of hypoglycaemia were seen. Mean euglycaemia scores of 96.2 (NHA) and 88.3 (IHA) from a maximum of 104 indicated marked ceiling effects. The NHA group scored better (P=0.016) on immediate visual memory.

**Table 5.2.** Results of memory tests. Effect of glycaemic condition is shown first for NHA and IHA subjects, and then glycaemic condition, hypoglycaemia awareness and glycaemia-awareness (G-A) interaction for all subjects combined. Data are mean (SD).

| | NHA subjects alone | | | IHA subjects alone | | | All subjects | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Glycaemia | | Awareness | | G-A interaction | |
| Test | Eu | Hypo | P | Eu | Hypo | P | Eu | Hypo | $\eta^2$ | P | $\eta^2$ | P | $\eta^2$ | P |
| **Prospective memory** | | | | | | | | | | | | | | |
| Immediate | 11.4 (3.9) | 10.1 (4.9) | 0.121 | 15.4 (4.2) | 13.3 (4.9) | **0.013** | 13.2 (4.4) | 11.6 (5.1) | 0.255 | **0.004** | 0.185 | **0.018** | 0.020 | 0.462 |
| **AVLT** | | | | | | | | | | | | | | |
| Immediate | 51.8 (7.8) | 45.1 (9.4) | **0.003** | 53.1 (12.5) | 50.3 (9.9) | 0.257 | 52.4 (10.0) | 47.4 (9.8) | 0.268 | **0.003** | 0.038 | 0.303 | 0.058 | 0.198 |
| Delayed | 10.0 (2.4) | 8.3 (2.7) | **0.012** | 10.9 (3.7) | 9.3 (3.3) | **0.008** | 10.4 (3.1) | 8.7 (3.0) | 0.373 | **0.000** | 0.033 | 0.334 | 0.000 | 0.927 |
| % retained | 79.9 (18.7) | 77.1 (18.3) | 0.530 | 81.7 (16.8) | 75.5 (18.9) | 0.104 | 80.7 (17.6) | 76.4 (18.3) | 0.077 | 0.138 | 0.000 | 0.981 | 0.011 | 0.578 |
| **Logical memory** | | | | | | | | | | | | | | |
| Immediate | 15.6 (3.5) | 13.1 (3.3) | **0.011** | 17.3 (3.9) | 16.3 (4.7) | 0.223 | 16.4 (3.7) | 14.5 (4.3) | 0.233 | **0.007** | 0.157 | **0.030** | 0.053 | 0.221 |
| Delayed | 13.1 (3.7) | 11.0 (3.4) | **0.010** | 15.8 (4.5) | 13.8 (4.3) | **0.047** | 14.3 (4.2) | 12.2 (4.0) | 0.315 | **0.001** | 0.155 | **0.031** | 0.001 | 0.898 |
| % retained | 84.2 (16.8) | 83.4 (16.3) | 0.903 | 89.6 (11.2) | 84.2 (14.4) | 0.223 | 86.6 (14.6) | 83.8 (15.3) | 0.022 | 0.431 | 0.027 | 0.385 | 0.013 | 0.548 |
| **Visual memory** | | | | | | | | | | | | | | |
| Immediate | 96.2 (8.0) | 96.4 (7.8) | 0.890 | 88.3 (15.0) | 89.3 (12.7) | 0.746 | 92.7 (12.1) | 93.3 (10.7) | 0.005 | 0.707 | 0.190 | **0.016** | 0.002 | 0.816 |
| Delayed | 82.8 (21.3) | 77.5 (25.0) | 0.128 | 81.3 (20.9) | 85.1 (17.3) | 0.277 | 82.1 (20.9) | 80.9 (22.0) | 0.004 | 0.745 | 0.007 | 0.669 | 0.116 | 0.066 |
| % retained | 85.4 (19.6) | 80.0 (24.1) | 0.122 | 91.9 (16.2) | 94.9 (11.9) | 0.570 | 88.3 (18.2) | 86.6 (20.8) | 0.006 | 0.675 | 0.099 | 0.090 | 0.069 | 0.162 |

## 5.4 Discussion

### 5.4.1 Prospective memory

Performance on the novel test of prospective memory was impaired significantly during the hypoglycaemia studies and, as learning always took place during euglycaemia, the results indicate that recall had been disrupted by hypoglycaemia. The effect size of hypoglycaemia on prospective memory scores ($\eta^2$) was similar to those for the conventional memory tests, which suggests that it is a similarly sensitive measure of hypoglycaemic memory dysfunction. The prospective memory task is also intuitively more reflective of memory in daily life, where list-learning is rarely relevant. In the paper upon which it was based, performance on the video task correlated well with performance on the same task in a real-life setting [4]. The data suggest that people with diabetes may suffer inability to act on previously-made plans, and thus effectively to organise aspects of their daily life, as a consequence of hypoglycaemia.

Some limitations of the prospective memory task must be considered [4]. Firstly, subjects are unable to explore the environment to obtain further cues if they suspect that an action is to be performed at a location, but cannot fully remember it. Secondly, they are unable to retrace their steps if an action is recalled after the location has been passed. Thirdly, to make the task sufficiently difficult, more items are given than subjects would attempt to remember in real life. Fourthly, subjects are unable to prioritize items and plan their journey. These points reflect the fact that in real life, prospective memory performance is not simply determined by accuracy of response to cues, but is also dependent on organisational and other strategies. Thus, the effect of hypoglycaemia on real-life prospective memory may be more or less than suggested by the present study, depending on whether these strategies are themselves sensitive or resistant to hypoglycaemia.

Before extrapolating to daily life in diabetes, the magnitude of the impairment associated with hypoglycaemia must also be considered. The mean score during hypoglycaemia was approximately 12% lower than during euglycaemia. Another measure is Cohen's d – the difference in means divided by the pooled standard deviation – which in this case

is 0.34, or half-way between a 'small' and 'medium' effect according to Cohen's conventions [16]. By both measures, the impairment of prospective memory due to hypoglycaemia was similar to (but slightly less than) the impairment for the AVLT and logical memory tasks (score reduction 10-16%, Cohen's d 0.47-0.60). These deteriorations themselves are much smaller than those reported by Sommerfield *et al*, which ranged from 15% to 55% at a similar level of hypoglycaemia [17,18]; the difference is hard to explain, but may be because both learning and recall occurred during hypoglycaemia in that study. For comparison, performance on the non-memory tasks reported in Chapter 6 deteriorated by 9-19% during hypoglycaemia.

It is also possible that poorer performance during hypoglycaemia was caused by impairment of cognitive functions other than memory. The video required sustained attention for around 10 minutes, and attention has been shown to be impaired during hypoglycaemia [19,20]. Deterioration of visual information processing may have reduced subjects' ability to glean information from the video [21,22]. Alternatively, the results may have resulted from a general effect on cognition, as very few cognitive abilities that have been tested during hypoglycaemia have not shown deterioration [23]. These distinctions may be neuropsychological rather than practical: whatever the mechanism, hypoglycaemia impaired the ability to act on information learned previously, which in everyday life would be perceived to be a memory problem.

Thus, it may be concluded that moderate hypoglycaemia causes a statistically-significant deterioration in performance on the prospective memory task, which mimics real life more closely than conventional tasks. This supports the extrapolation of laboratory data to practical advice for people with diabetes. The effect size was modest, though comparable to that seen for other memory and cognitive tasks.

### 5.4.2 Verbal and visual memory

Immediate recall performance for the two verbal memory tasks (AVLT and logical memory) was impaired significantly during hypoglycaemia, consistent with previous studies [17,18]. Delayed recall was always tested during euglycaemia, and it can be inferred that the significant impairment in delayed recall following hypoglycaemia

resulted from a hypoglycaemic effect on learning and/or consolidation. A 'hangover' effect of hypoglycaemia is unlikely to be responsible, as delayed recall was tested 50 minutes after restoration of euglycaemia. The only robust study to show residual cognitive impairment demonstrated this 20 minutes after hypoglycaemia [24]; earlier studies reported impaired cognitive function 45 to 90 minutes after hypoglycaemia [25,26,27,28,29,30], but each had a methodological deficiency such as failure to test statistical significance or absence of a euglycaemia control arm.

Correction of hypoglycaemia took approximately 20 minutes, the limiting factors being the continuing insulin infusion and the maximum infusion rate of hypertonic glucose solution. The possibility that hypoglycaemia impaired performance through an effect on consolidation in the immediate post-learning period cannot be excluded. However, on this basis it is also relatively unimportant to separate learning and immediate consolidation, as hypoglycaemia in the daily life of a person with diabetes will not be reversed more quickly. The practical conclusion that may be drawn from the verbal memory results is that hypoglycaemia impairs the learning of information so that it may be recalled at a later time. For example, if hypoglycaemia occurred while studying, the student would be advised to review material that had recently been examined.

Visual memory was not impaired significantly by hypoglycaemia, but with mean scores of 93% and 82% for immediate and delayed recall during euglycaemia, a ceiling effect was evident. Future work on this aspect of memory should employ tasks with a larger range of items, especially more difficult items.

### 5.4.3 Normal and impaired hypoglycaemia awareness

There was no evidence of a different effect of hypoglycaemia in IHA and NHA subjects. This may have been related to inadequate statistical power for this specific between-subjects analysis. However, with a total of 36 subjects and a repeated-measures design, the study was large by comparison with others in this research area. There are some limitations of the between-group comparisons. Firstly, we cannot exclude overlap, even though our method of assessing awareness has been shown to be a good predictor of severe hypoglycaemia at the group level [29]. Subjects with similar levels of

awareness could receive different classification as a result of answering with confidence or caution. Secondly, the two groups were not well matched for other characteristics which may have confounded the effect of hypoglycaemia on memory performance, particularly duration of diabetes, presence of microvascular complications and overall memory performance. For these reasons, the present study cannot exclude a potential difference between people with IHA and NHA.

An important consideration is that studies of this type, which compare group means using a powerful within-subjects design, do not have much power to examine individual differences in the effects of hypoglycaemia on cognition. There is, in our experience, consistent anecdotal evidence from both clinical practice and research to suggest that some individuals are much more susceptible to hypoglycaemia-induced cognitive dysfunction than others. To assess this experimentally is impractical: for this type of intensive study, an uncommonly large number of subjects would be required. However, given the possibility of inter-individual differences, practical recommendations to people with diabetes should not be too rigid.

### 5.4.4 Conclusions

In conclusion, the present study has confirmed that there is a disruptive effect of hypoglycaemia on both the specific processes of learning and recall, which is relevant to the behaviour of people with diabetes during daily life. The prospective memory test appears to be a sensitive measure of memory dysfunction during hypoglycaemia which may have greater ecological validity than conventional memory tests, and lends weight to the extrapolation of laboratory data to practical recommendations for people with diabetes.

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# Chapter 6

# The Effects of Acute Hypoglycaemia on Non-verbal Intelligence, and Accuracy and Speed of Visuospatial Cognitive Function

## 6.1 Introduction

### 6.1.1 Non-verbal intelligence

In earlier Edinburgh studies, McAulay *et al* reported that several facets of attention deteriorated significantly at an arterialized blood glucose level of 2.6 mmol/L in non-diabetic [1] and type 1 diabetic subjects [2]. Nonverbal intelligence was also measured using Raven's Progressive Matrices (RPM), and scores on this test were not significantly impaired by hypoglycaemia at either 20 minutes or completion of the test (with no time limit). This was unexpected, as various domains of cognitive function had consistently been impaired at this level of hypoglycaemia previously [3]. Additionally, RPM is acknowledged as among the best indicators of general fluid intelligence [4], and has a substantial correlation with working memory [5], which is exquisitely sensitive to hypoglycaemia [6]. The RPM consists of sequences of geometric designs governed by logical rules, which must be deduced, held in working memory and applied simultaneously to continue the sequence from a choice of answers [7]. Figure 6.1 gives an example of this type of problem.

As discussed in sections 2.2.3 and 2.2.4, it has been suggested that different mental functions have variable sensitivity hypoglycaemia. It is possible that the higher-level cognitive skills required for abstract problem-solving (as in the RPM) are resistant to hypoglycaemia. However, this contradicts a widely-accepted view that higher-level skills are more sensitive to hypoglycaemia than simple, repetitive cognitive or motor tasks [3].

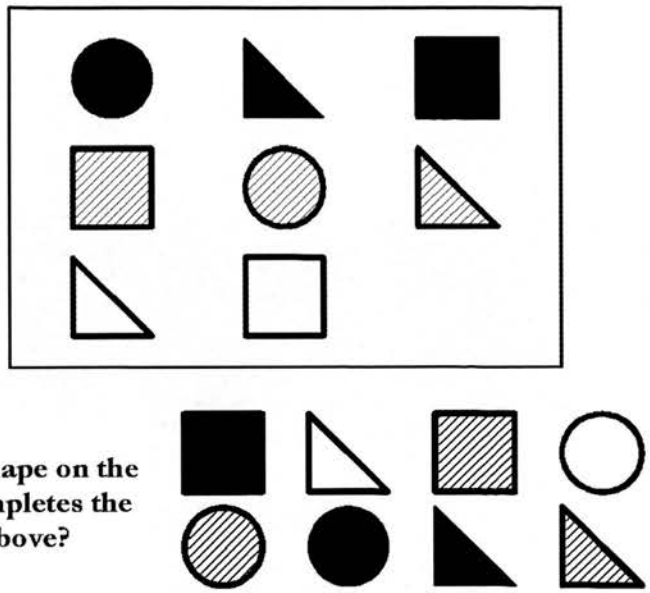


**Figure 6.1.** Illustrative (easy) example of problems in Raven's Progressive Matrices.

An alternative explanation is the possibility of a ceiling effect. It is assumed that if a subject finds a problem easy, then they have cognitive spare capacity, and any intervention that disturbs cognitive function must first eradicate the spare capacity before performance is reduced. Thus, an intervention that causes mild or moderate cognitive deterioration may not affect scores on easy tests. In McAulay *et al*'s studies, mean scores during euglycaemia were 82.5% in non-diabetic subjects [1] and 86.2% in diabetic subjects [2], which are high but probably not excessively high. However, a reasonable mean score may reflect a combination of very easy and harder problems. The problems in the RPM are progressively more difficult, but many are rather easy, and the subjects studied were generally of high intellectual ability. Figure 6.2 illustrates how the sensitivity of a test for cognitive decline may depend on the proportion of easy problems.

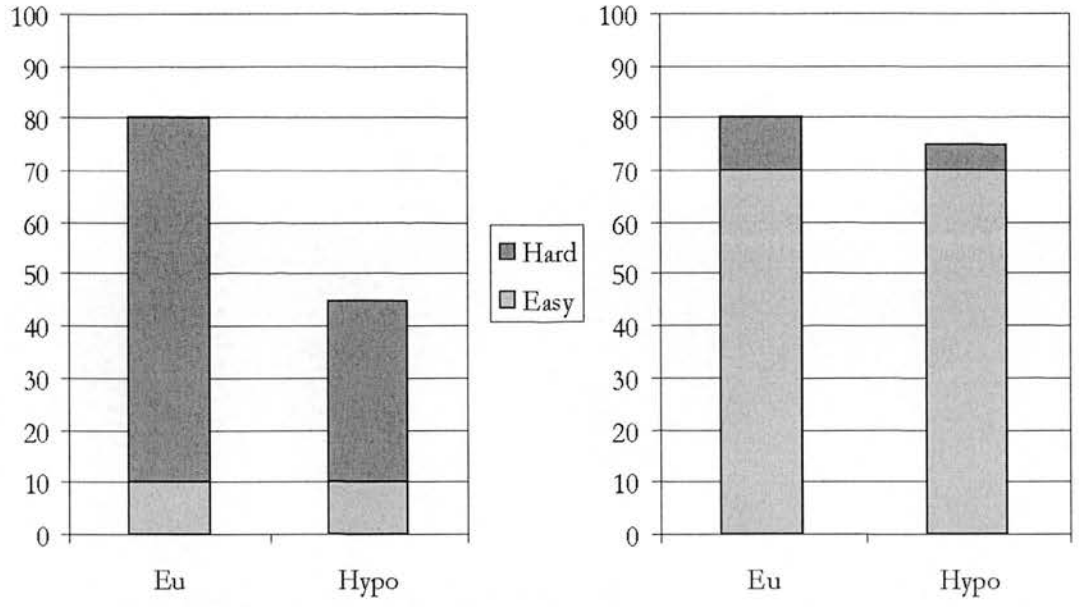


**Figure 6.2.** Hypothetical representation of the effect of hypoglycaemia on tests with a low proportion (left graph) and a high proportion (right graph) of easy problems. Performance on easy problems is assumed to be unaffected by hypoglycaemia; performance on hard problems is assumed to be reduced by 50%. Euglycaemia scores are identical for the two tests, but the test in the right graph is insensitive to hypoglycaemic cognitive dysfunction.

Thus, a key requirement was to repeat McAulay *et al*'s studies using similar but harder cognitive tests. Raven's Advanced Progressive Matrices (RAPM) uses harder problems of the same type as RPM, and was ideally suited to this function. The first primary aim of this study was to determine whether performance on RAPM is impaired by hypoglycaemia.

### 6.1.2 Speed vs Accuracy

As briefly discussed in section 2.2.2, although many experiments have been reported as showing cognitive impairment during hypoglycaemia, for the vast majority it is more correct to say that they have shown cognitive slowing. Table 2.1 listed the most commonly used cognitive function tests. For Trail Making B, the Stroop ink colour tasks and the reaction time variants, the score is the time to complete a certain number of tasks. Finger tapping, verbal fluency and Digit Symbol Substitution are all scored as number of tasks completed in a set time; since the individual tasks are very easy, what is

effectively measured is again speed. In Table 2.1, only the digit and word recall scores are not explicitly timed, and these are among the few tasks that have shown reduced accuracy of cognition during hypoglycaemia.

In daily life, both speed and accuracy are relevant to most tasks. It would be of practical interest to know whether hypoglycaemia primarily affects speed or accuracy of cognition. The second primary aim of this study was to determine the effects of hypoglycaemia on performance on a test scored for both accuracy and speed. A computer-based maze task was used for this.

## 6.2 Methods

### 6.2.1 Experimental design and methodology

This study was embedded within the study of memory consolidation reported in chapter 4, and the subject details and experimental methodology are described in full there. In brief, sixteen non-diabetic volunteers, aged 20-44 years, were studied. Each subject underwent two hyperinsulinaemic glucose clamps, with experimental states of euglycaemia and hypoglycaemia.

Initially, blood glucose was stabilized at 4.5 mmol/L in all cases, and at this point subjects were exposed to the face and word lists for the study described in chapter 4. Blood glucose levels were then either lowered to 2.5 mmol/L (hypoglycaemia) and maintained at that level for one hour, or maintained at 4.5 mmol/L for the same length of time. Subjects were not informed of the experimental condition. During the experimental hour, subjects completed the cognitive function tests detailed below. Euglycaemia was then restored, and the clamp discontinued. Events after this point (fMRI scanning, as described in chapter 4) have no bearing on the present study.

### 6.2.2 Intelligence and other cognitive function tests

All subjects completed the National Adult Reading Test (NART) at the start of their first session. In the NART, the subject is presented with a list of words that are used very infrequently in everyday spoken English, and is asked to read them aloud. Pronunciation is scored with reference to an audio tape of correct pronunciation. Performance on this test has been shown to correlate very highly with best-ever Wechsler Adult Intelligence Test scores [8].

The tests that were completed during euglycaemia-hypoglycaemia were Raven's Advanced Progressive Matrices (RAPM) [9], the Alice Heim 5 test (AH5) [10], Trail-Making B (TMB) and the Digit Symbol Substitution Test (DSST).

The Alice Heim 5 test (AH5) similarly requires identification and application of simultaneous patterns to complete sequences, although these may be verbal, numerical or geometric. Paper working is permitted for AH5 but not for RAPM. Parallel versions of the RAPM and AH5 were created by separating odd- and even-numbered items, with Set 1 and Set 2 of RAPM being combined; thus, two batteries were created, each consisting of half of the RAPM questions and half of the AH5 questions. Subjects were limited to 20 minutes for RAPM questions, and 20 minutes for AH5 questions.

The TMB test was performed on an Apple Newton handheld computer with a touch-sensitive screen. The DSST test was performed using paper and pen.

### 6.2.3 Maze task

This task was developed and supplied by Brian Tiplady of Penscreen Ltd. An Apple Newton handheld computer with a 5 by 8 inch touch-sensitive screen was loaded with several different maze designs, of which one example is shown in Figure 6.3. Each maze had only one path without junctions or dead ends, and hence did not require any route-finding. Subjects were given the exact instruction "Draw the route from the entrance to the exit as quickly and as accurately as you can, staying in the white areas and avoiding the black walls." If required, a minimum of demonstration was provided with no guidance as to whether speed or accuracy was more important.

During each study, the subject completed mazes on five occasions: the first two during initial euglycaemia, the third at the start and the fourth at the end of the experimental hour, and the fifth when euglycaemia was restored after the experimental hour. On each occasion the subject completed two mazes: one was the same across the five occasions (allowing a learning effect) and the other varied.

Each maze task yielded the following data: completion time, accuracy (percentage of the trail within the white areas) and number of errors (number of occasions the black walls were touched or crossed).

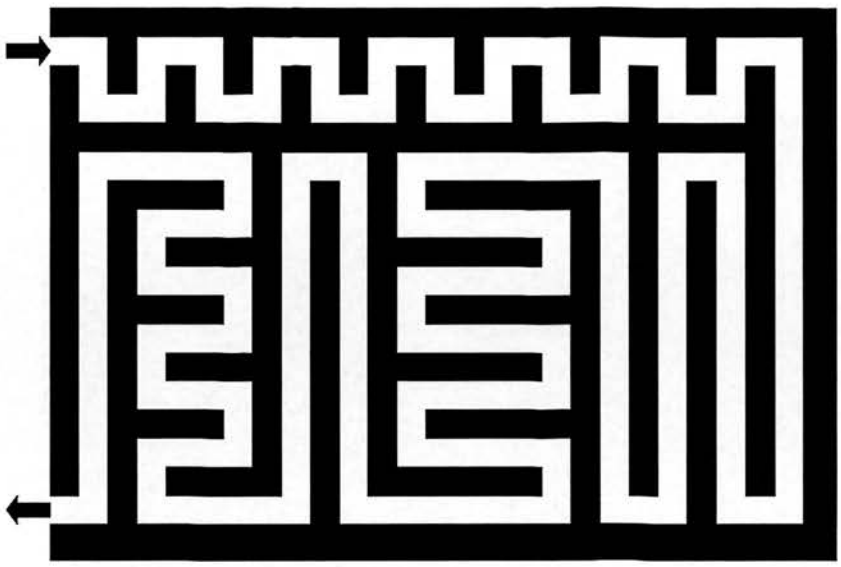

**Figure 6.3.** Example of maze task.

### 6.2.4 Data analysis

Data were analysed using SPSS 11.0. Euglycaemia-hypoglycaemia comparisons were made by repeated-measures analysis of variance (ANOVA). The order of euglycaemia-hypoglycaemia and the two cognitive test batteries were counterbalanced, and included as between-subjects factors in repeated measures ANOVA. The threshold for statistical significance was set at P=0.05.

## 6.3 Results

There were no significant effects of order of glycaemic condition or test battery on any test.

### 6.3.1 Intelligence and other cognitive function test results

The mean NART score was 40, approximating to a WAIS-R Full Scale IQ score of 118.

Scores on other tests are given in Table 6.1. Performance on all tests other than TMB are given as mean number of correct answers (SD); TMB performance is reported as completion time in seconds (SD), i.e. shorter completion time represents better performance. Maximum possible scores were 36 for AH5, and 24 for RAPM. Effect sizes ($\eta^2$) and P-values for euglycaemia-hypoglycaemia comparisons are given in Table 6.1.

Performance on TMB, DSST and RAPM deteriorated significantly during hypoglycaemia. There was a trend towards deterioration on AH5, which failed to achieve statistical significance (P=0.057).

| Test | Euglycaemia | Hypoglycaemia | P | $\eta^2$ |
|---|---|---|---|---|
| RAPM | 18.9 (3.1) | 16.5 (3.5) | 0.007 | 0.465 |
| AH5 | 15.6 (5.6) | 13.9 (5.0) | 0.057 | 0.269 |
| TMB | 38.8 (8.2) | 46.3 (16.1) | 0.037 | 0.339 |
| DSST | 87.7 (12.5) | 79.4 (9.3) | 0.019 | 0.409 |

**Table 6.1.** Mean (SD) cognitive function scores during euglycaemia and hypoglycaemia.

The statistical power of the study was determined *post hoc* from these data. For 80% power to detect a moderate effect size of 0.5 standard deviations at P<0.05, a sample size of 41 subjects would be required for the AH5 test. The observed power was 57%,

so in hindsight the sample size was significantly too small to be sure of detecting a result, and the negative result for AH5 cannot be taken as evidence of no effect.

To assess whether hypoglycaemia had any effect on strategy, the numbers of RAPM and AH5 items attempted and the proportions correctly answered were analysed. Results are given in Table 6.2. For RAPM and AH5, both number of items attempted and proportion correct were slightly lower under hypoglycaemia, but these differences were not statistically significant.

| | Euglycaemia | Hypoglycaemia | P |
|---|---|---|---|
| **RAPM** | | | |
| Attemped | 22.0 (3.0) | 20.8 (2.6) | 0.189 |
| Percent correct | 85.1 (11.3) | 80.7 (13.2) | 0.406 |
| **AH5** | | | |
| Attempted | 23.1 (7.3) | 21.3 (6.8) | 0.364 |
| Percent correct | 67.3 (16.0) | 66.2 (14.3) | 0.750 |

**Table 6.2.** Mean (SD) number of questions attemped and mean (SD) percentage correct, with P for euglycaemia-hypoglycaemia comparison.

### 6.3.2 Maze results

Accuracy scores and completion times are shown in Table 6.3, and are provided separately for the five different time points and for the fixed and variable mazes. Only one P-value crosses the standard 0.05 threshold – this is for completion time of the fixed mazes during the recovery period. It is not impossible that there should be a hangover effect from hypoglycaemia, but this seems unlikely in the absence of any effect during hypoglycaemia. Table 6.3 contains 20 statistical comparisons, and if any one is to be accepted then the P threshold must be set at 0.0025. Thus, no statistically significant effects of hypoglycaemia on either accuracy or speed are demonstrated.

| | Accuracy Eu | Accuracy Hypo | P | Time Eu | Time Hypo | P |
|---|---|---|---|---|---|---|
| **Fixed maze** | | | | | | |
| **Baseline 1** | 96.1 (4.2) | 97.4 (3.9) | 0.393 | 25.4 (12.8) | 29.4 (14.9) | 0.382 |
| **Baseline 2** | 97.4 (1.9) | 97.8 (2.4) | 0.542 | 23.8 (7.1) | 23.2 (6.5) | 0.633 |
| **Exp start** | 98.3 (2.1) | 97.8 (2.1) | 0.596 | 22.1 (5.3) | 24.0 (8.0) | 0.231 |
| **Exp end** | 97.1 (2.9) | 96.7 (3.0) | 0.684 | 21.4 (6.7) | 25.2 (7.8) | 0.168 |
| **Recovery** | 96.7 (2.8) | 97.4 (3.2) | 0.368 | 21.7 (6.0) | 25.8 (8.1) | **0.049** |
| **Variable mazes** | | | | | | |
| **Baseline 1** | 97.2 (3.3) | 97.9 (2.5) | 0.345 | 26.3 (13.2) | 24.3 (11.1) | 0.613 |
| **Baseline 2** | 96.3 (5.6) | 97.4 (2.1) | 0.309 | 21.9 (6.2) | 21.0 (6.3) | 0.478 |
| **Exp start** | 97.0 (2.8) | 97.3 (3.2) | 0.687 | 22.7 (10.2) | 24.4 (6.8) | 0.609 |
| **Exp end** | 97.6 (2.9) | 97.0 (3.4) | 0.567 | 20.9 (6.2) | 22.2 (5.4) | 0.481 |
| **Recovery** | 97.5 (2.7) | 96.2 (5.2) | 0.344 | 19.6 (6.5) | 20.1 (4.8) | 0.404 |

**Table 6.3.** Accuracy (SD) and completion time (SD) scores for maze task. Accuracy is % of trail within boundaries, time is in seconds. P for euglycaemia-hypoglycaemia comparisons.

It is possible that the maze task suffered from a ceiling effect, although this cannot be determined from data in the present study. Mean maze accuracy scores during euglycaemia were approximately 97%, but this does not have the same meaning as a 97% correct score on a test like RAPM. This score indicates that 97% of the length of the trail was drawn within the boundaries, which may or may not be a good achievement. (Staying on the road 97% of the time could not be considered good driving!). Population norms are not available for comparison with the performance of the high-ability subjects in the present study.

## 6.4 Discussion

Hypoglycaemia impaired performance on Raven's Advanced Progressive Matrices in the present study. These results are at odds with findings from the earlier studies by McAulay *et al* using the simpler Raven's Progressive Matrices [1,2]. From those earlier studies, the scores after 20 minutes (which are directly comparable to scores in the present study) were 80.7% in non-diabetic and 83.5% in diabetic subjects during euglycaemia, and no significant decline was seen during hypoglycaemia. In the present study, the mean score on Raven's Advanced Progressive Matrices during euglycaemia was very similar at 79%, but performance declined with clear statistical significance during hypoglycaemia. The effect size of hypoglycaemia on RAPM was substantial, amounting to approximately three-quarters of a standard deviation. A plausible explanation for these disparate results is that in intelligent subjects, a ceiling effect rendered the RPM relatively insensitive to the effects of hypoglycaemia.

Mean scores on the Alice Heim 5 test fell from 43% during euglycaemia to 39% during hypoglycaemia, but this failed to achieve statistical significance. In retrospect, the study was underpowered, but other possible contributory factors are of interest. The AH5 is harder than RPM or RAPM, and there may have been a floor effect. Alternatively, the different results for RAPM and AH5 may reflect a difference in their administration: paper working is permitted for the AH5, but not for the RAPM. Holding and applying the AH5 rules entirely in working memory is demanding, and a demanding working memory task has been shown to be exceptionally sensitive to moderate hypoglycaemia [6].

In combination, these results indicate that moderate hypoglycaemia can impair general fluid intelligence, but it is necessary to use a test appropriate to the ability level of the participants. Research volunteers tend to be more able than the population they are intended to represent, and the ceiling effect may be common in hypoglycaemia studies. Care should be taken to avoid both ceiling and floor effects, and, while general consensus on cognitive testing is desirable, a single battery of cognitive tests for all subjects may be inappropriate [11].

The RAPM and AH5 results did not indicate whether speed or accuracy of cognition is primarily affected during hypoglycaemia. The maze task was scored for both accuracy and speed, and so might have demonstrated a tendency for one to be sacrificed to preserve the other under adverse conditions. However, there was no evidence of hypoglycaemic deterioration on any measure of performance on this task. The cognitive demands imposed by the maze task are clearly different from those imposed by RAPM: intuitively, the maze task engages hand-eye coordination and fine motor control, whereas RAPM requires the simultaneous development and testing of abstract rules, with a heavy load on working memory and visuo-spatial representation. It is possible that these differences explain why the latter was sensitive to hypoglycaemia and the former was not. However, a more recent study has clearly demonstrated that equivalent hypoglycaemia induces significant impairment in performance on various similar psychomotor tasks in non-diabetic adults [12]. It is more likely that the maze task (like RPM) was too easy to be discriminatory, and the question of speed versus accuracy remains unanswered.

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# Chapter 7

# Symptoms of Hyperglycaemia

## 7.1 Introduction

People with diabetes are commonly exposed to fluctuations in blood glucose into the hypo- and hyperglycaemic ranges. The symptoms of hypoglycaemia have been described in detail, and their generation has been studied using different approaches [1]. The statistical technique of factor analysis has been used to identify discrete groups, or domains, of symptoms, which co-segregate; that is, symptoms which tend to be reported by the same people. This technique has been applied to symptoms reported during experimental hypoglycaemia [2], or derived from population surveys [3,4]. The potential value of this analysis is that symptom groups identified may represent underlying physiological processes. In the case of hypoglycaemia symptoms, two clear groups apparently representing *neuroglycopenic* and *autonomic* symptoms emerged from factor analysis [1,4], which suggests that the symptoms may be generated by separate physiological mechanisms. This is consistent with studies of experimentally-induced hypoglycaemia with different forms of pharmacological blockade [5,6], and studies in people with diabetes and impaired hypoglycaemia awareness [7,8,9,10], in whom autonomic but not neuroglycopenic symptoms are diminished or lost.

Symptoms of hyperglycaemia are often present at the time of diagnosis of diabetes, and the classical osmotic symptoms include thirst, polyuria and nocturia [11]. However, there are few experimental studies of the symptoms of hyperglycaemia. When acute hyperglycaemia was induced by a hyperinsulinaemic glucose clamp technique, only one symptom (the need to urinate) was positively associated with this state [12]. A recent Edinburgh study examined mood (rather than symptoms) during hyperglycaemia in subjects with type 2 diabetes, again using a hyperinsulinaemic glucose clamp, and reported that hyperglycaemia was associated with reduced energetic arousal and increased sadness and anxiety [13]. In a pair of earlier studies, many subjects reported

symptoms which correlated positively with blood glucose concentrations [14], but these symptoms varied widely between subjects, and subsequently were found to be unstable over time [15]. A study of all symptoms in type 2 diabetes listed thirst, dry mouth, polyuria and polydipsia as symptoms of hyperglycaemia, but this study did not focus specifically on acute hyperglycaemia [16]. However, it is possible that symptoms experienced during experimentally-induced hyperglycaemia are not representative of symptoms experienced by people with diabetes during daily life.

From clinical experience, people with insulin-treated diabetes recognize various unpleasant symptoms that are associated with transient hyperglycaemia, which may prompt them to measure blood glucose and modify their treatment. The perception of the development of hyperglycaemic symptoms may assist the individual to improve glycaemic control, and the early treatment of elevated blood glucose may prevent progression to metabolic decompensation. The principal aims of the present study were to determine which symptoms of hyperglycaemia are reported commonly by people with insulin-treated diabetes, and to ascertain whether any underlying structure could be identified to assist with the classification of the symptoms. Secondary aims were to determine whether the intensity of, and glycaemic threshold for, symptoms of hyperglycaemia are related to the co-existing state of hypoglycaemia awareness, the quality of glycaemic control, the duration of insulin therapy, and concomitant drug therapy.

## 7.2 Methods

### 7.2.1 Pilot study

A pilot study was performed to identify possible symptoms of hyperglycaemia. Seventy patients with insulin-treated diabetes were asked to report, in an open-ended manner, symptoms which they associated with a high blood glucose. Their reports were grouped together as symptoms when identical wording was used, or when it was clear that the same sensation was being described. A total of 25 different symptoms were reported, 17 of which were reported by more than one person (Table 7.1). In formulating the symptom questionnaire, one additional symptom (an increase in salivation) which had been reported by more than one subject in a previous study [14] was incorporated. These 18 symptoms were used to compile the symptom questionnaire for the main study. Patients participating in the pilot study did not take part in the main study.

| |
|---|
| Blurred vision |
| Thirst |
| Nausea |
| Not feeling right |
| Weakness |
| Dry mouth |
| Irritability |
| Light-headedness |
| Need to urinate |
| Headache |
| Restlessness |
| Sweet or funny taste |
| Stomach ache |
| Poor concentration |
| Dizziness |
| Feeling tense |

**Table 7.1.** Symptoms associated with hyperglycaemia reported by more than one individual in pilot study.

### 7.2.2 Subjects

To provide a robust solution using principal components analysis, the most cautious estimate of the number of subjects required is 10 times the number of variables under examination [17]. With 18 symptoms in the questionnaire, 180 subjects should suffice. A target of 400 subjects was set to provide a large safety margin.

Approval for the study was granted by the local research ethics committee. People were recruited during their routine attendances at diabetes outpatient clinics in the Department of Diabetes at the Royal Infirmary of Edinburgh. Patients were eligible if they had received full insulin therapy for at least one year, performed self-monitoring of blood glucose (irrespective of frequency), and were capable, in the opinion of the investigator, of answering the questionnaire. Patients using insulin at bedtime only were excluded, but any full-time regimen (twice daily injections or more often) was permitted. There were no restrictions on other drug therapies. All consecutive patients who met the inclusion criteria and who consented to take part were recruited until the target of 400 had been achieved. Characteristics of the participants are shown in Table 7.2. Written informed consent was obtained before the questionnaire was applied.

| | Total | Type 1 | Type 2 |
|---|---|---|---|
| **n** | 400 | 288 | 112 |
| **Male (%)** | 229 (57.3) | 173 (60.1) | 57 (50.9) |
| **Median age, in years (range)** | 49 (18-84) | 41 (18-79) | 66 (35-84) |
| **Median duration of diabetes, in years (range)** | 14 (1-59) | 15 (1-59) | 12 (4-36) |
| **Median duration of insulin therapy, in years (range)** | 11 (1-59) | 15 (1-59) | 4 (1-33) |
| **Mean $HbA_{1c}$ (SD) (%)** | 8.6 (1.3) | 8.6 (1.4) | 8.7 (1.3) |

**Table 7.2.** Characteristics of insulin-treated diabetic subjects completing symptom questionnaire.

### 7.2.3 Questionnaire

Each subject completed the questionnaire once. Subjects provided estimates, based on prior experience, of the intensity with which they usually felt each symptom during periods of hyperglycaemia ("when your blood glucose level is high"). Hyperglycaemia was not defined more explicitly in terms of blood glucose concentration. Estimates of symptom intensity were recorded on a visual scale from 1 ("not at all") to 7 ("very strongly"). The participants were asked to indicate the approximate level of blood glucose at which they would expect to experience hyperglycaemic symptoms, based on previous blood glucose testing, and to describe any other symptoms which they associated with hyperglycaemia. In addition, they were asked to rate their symptomatic awareness of hypoglycaemia on a visual scale from 1 (always aware) to 7 (never aware) [9]. Duration of diabetes and duration of insulin therapy, details of concomitant drug therapy, and most recent glycated haemoglobin ($HbA_{1c}$) measurements were recorded. Glycated haemoglobin was measured by high performance liquid chromatography (BioRad Variant 2, Hemel Hempstead, UK; local non-diabetic range 5.0-6.5%).

### 7.2.4 Principal components analysis

Data were analysed using SPSS 11.0 (SPSS, Chicago, IL). Correlations among the intensity ratings of the 18 symptoms were examined using principal components analysis. The monograph by Child provides an accessible explanation of this technique [17]. Briefly, the procedure examines the correlations among measured variables to determine whether there are groups of variables which occur together. This could be done informally: it might, for example, be apparent from inspection of correlation coefficients that there were three groups of symptoms, such that symptoms were strongly correlated with other symptoms in the same group, but only weakly correlated with symptoms in other groups. In this hypothetical case, the entire data set could be reduced to three 'factors', each representing a group of variables from the original data set. If the within-group correlations had been high, then this process would greatly simplify the data set with only minimal loss of information. The value of this technique in examining symptoms arises from the fact that symptom grouping may hint at underlying physiological processes. For example, in previous studies, the symptoms of

confusion, odd behaviour, inability to concentrate, drowsiness and difficulty speaking were highly correlated, and were interpreted as the effects of neuroglycopenia; whereas hunger, sweating, trembling, anxiety and a pounding heart formed a separate, highly correlated group, which was interpreted as the effects of autonomic activation [2,3,4].

Principal components analysis (PCA), sometimes referred to as factor analysis, is a statistical tool for extracting components which simplify the data, while retaining as much information as possible. Technically, components are computed first, and undergo a mathematical transformation called rotation to yield more easily interpretable 'factors'. Although there is a technical difference between 'component' and 'factor', for the sake of clarity 'factor' is used here. The *variance* explained by a factor indicates the amount of information from the original data which is contained within the computed factor. PCA initially generates the same number of components as the number of symptoms under examination, but most explain very little variance. Only components which usefully simplify the data are considered significant, and these are determined by selection of those which are outliers on a plot of variance (the 'scree slope criterion') and/or those which explain more variance than a single variable from the original data (the 'eigenvalues greater than one criterion').

PCA factors are mathematical constructs, and their interpretation relies on *factor loadings*, which are the correlations between the original symptom scores and the extracted factors. Factors are considered to represent those symptoms with which they have moderate to high correlations. A cut-off for the correlation coefficient of 0.3 to 0.4 is generally applied [17]. The internal consistency of the grouping of symptoms suggested by this method may be confirmed by computing Cronbach's Alpha statistic.

### 7.2.5 Other statistical methods

Mean symptom intensity scores for each subject were calculated as the arithmetical means of the scores for each variable. Associations between these scores and other characteristics of the participants were investigated with Spearman rank correlations, Mann-Whitney U tests and linear regression techniques. Scatter plots were examined for correlations to exclude artefacts from outlying cases.

## 7.3 Results

### 7.3.1 Symptom endorsements

Of the 400 respondents, 361 (90.2%) identified one or more of the 18 symptoms as associated with hyperglycaemia. The five most commonly endorsed symptoms were 'thirst', 'dry mouth', 'not feeling right', 'need to urinate' and 'tiredness'. The absolute endorsement frequencies (i.e. any response greater than 'not at all') and the mean scores (1-7) for each symptom are given in Table 7.3.

| Symptom | Mean score (SD) | Absolute endorsement (%) |
|---|---|---|
| Thirst | 4.06 (2.05) | 79.5 |
| Dry mouth | 4.09 (2.09) | 78.7 |
| Not feeling right | 3.80 (2.08) | 75.6 |
| Need to urinate | 3.71 (2.35) | 64.5 |
| Tiredness | 3.30 (2.23) | 61.5 |
| Irritability | 2.80 (2.18) | 48.2 |
| Poor concentration | 2.59 (1.96) | 47.9 |
| Weakness | 2.36 (1.85) | 43.2 |
| Sweet/funny taste | 2.53 (2.09) | 41.0 |
| Restlessness | 2.30 (1.88) | 40.2 |
| Feeling tense | 2.19 (1.82) | 38.0 |
| Headache | 2.12 (1.81) | 34.1 |
| Blurred vision | 1.81 (1.45) | 29.4 |
| Light headed | 1.80 (1.53) | 26.9 |
| Nausea | 1.80 (1.54) | 26.6 |
| Dizziness | 1.70 (1.50) | 22.4 |
| Stomach ache | 1.42 (1.17) | 13.9 |
| Increased saliva | 1.34 (1.07) | 11.9 |

**Table 7.3**. Mean intensity scores and absolute endorsement rates for each symptom.

### 7.3.2 Principal components analysis

Two of the 18 symptoms ('stomach ache' and 'increased salivation') had absolute endorsement levels below 20%. Weakly endorsed variables cannot be reliably

interpreted by PCA, and these symptoms were excluded from subsequent analysis. PCA was performed on symptoms data from the 361 subjects who reported any hyperglycaemic symptoms using the remaining 16 symptoms. The first unrotated principal component has strong loadings (0.47 or greater) from all symptoms (Table 7.4), and accounts for 37.7% of total variance. The high loadings and high internal reliability (Cronbach's Alpha 0.89) indicate that it is a general hyperglycaemic symptoms component. Because of this, rotation was performed using the Direct Oblimin method, which permits factors to be correlated (non-orthogonal).

A four-factor solution was appropriate, based both on eigenvalues greater than one, and on scree-slope inspection (Figure 7.1). Variances accounted for by each factor, and loadings from each of the 16 symptoms, are shown in Table 7.4. Factor loadings greater than 0.35 were considered significant. The first factor ('feeling tense', 'irritability', 'restlessness' and 'poor concentration') contains symptoms which appear to have in common some aspect of *mental agitation*. The second factor ('thirst', 'dry mouth', 'need to urinate', 'not feeling right', 'sweet or funny taste' and 'weakness') mainly represents symptoms resulting from the *osmotic* effects of hyperglycaemia. The third factor ('dizziness', 'blurred vision', 'light-headedness' and 'weakness') contains symptoms similar to those designated *neuroglycopenic* in previous studies of hypoglycaemic symptoms using factor analysis [2,3,4], although *neurological* may be a better nomenclature in the present context. The fourth factor has strong loadings from 'headache', 'nausea' and 'sweet or funny taste'. Headache and nausea also appeared as a factor (labelled *malaise*) in hypoglycaemia studies [2,3,4].

The total variance explained by the four-factor model is 62.9%. The factors are correlated, and so the sum of the individual variances exceeds this total. Cronbach's Alpha was calculated as a measure of internal consistency for the general hyperglycaemia factor and each of the four rotated factors (Table 7.4). All except the malaise factor had high internal consistency coefficients (> .7).

| | First unrotated principal component | Rotated factors 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| **Variance** (%) | 33.7 | 24.6 | 21.5 | 21.2 | 13.3 |
| **Cronbach Alpha** | .86 | .79 | .79 | .74 | .51 |
| **Symptom** | **Component loadings** | **Pattern matrix loadings** | | | |
| **Thirst** | .51 | -.13 | **-.89** | .03 | -.03 |
| **Dry mouth** | .57 | -.10 | **-.82** | .03 | .14 |
| **Not feeling right** | .70 | .01 | **-.41** | .14 | .15 |
| **Need to urinate** | .44 | .08 | **.73** | -.16 | .05 |
| **Tiredness** | .62 | **.36** | .20 | .33 | -.07 |
| **Irritability** | .65 | **.80** | .05 | -.04 | .08 |
| **Poor concentration** | .67 | **.56** | .09 | .29 | -.07 |
| **Weakness** | .61 | .18 | **.40** | **.47** | -.26 |
| **Sweet/odd taste** | .57 | .14 | **.42** | -.06 | **.43** |
| **Restlessness** | .63 | **.78** | -.03 | -.06 | .13 |
| **Feeling tense** | .58 | **.87** | -.11 | -.03 | -.04 |
| **Headache** | .51 | .08 | .05 | .07 | **.73** |
| **Blurred vision** | .45 | -.09 | -.06 | **.76** | .08 |
| **Light headedness** | .62 | .08 | -.03 | **.73** | .14 |
| **Nausea** | .48 | .01 | .02 | .17 | **.69** |
| **Dizziness** | .59 | .03 | -.06 | **.83** | .07 |

**Table 7.4.** Results of principal components analysis of symptom intensity scores, with Direct Oblimin rotation.

| Factor 1 Mental agitation | Factor 2 Osmotic | Factor 3 Neurological | Factor 4 Malaise |
|---|---|---|---|
| Feeling tense | Thirst | Dizziness | Headache |
| Irritability | Dry mouth | Blurred vision | Nausea |
| Restlessness | Need to urinate | Light headedness | |
| Poor concentration | | | |

**Table 7.5.** Symptoms loading strongly onto PCA factors.

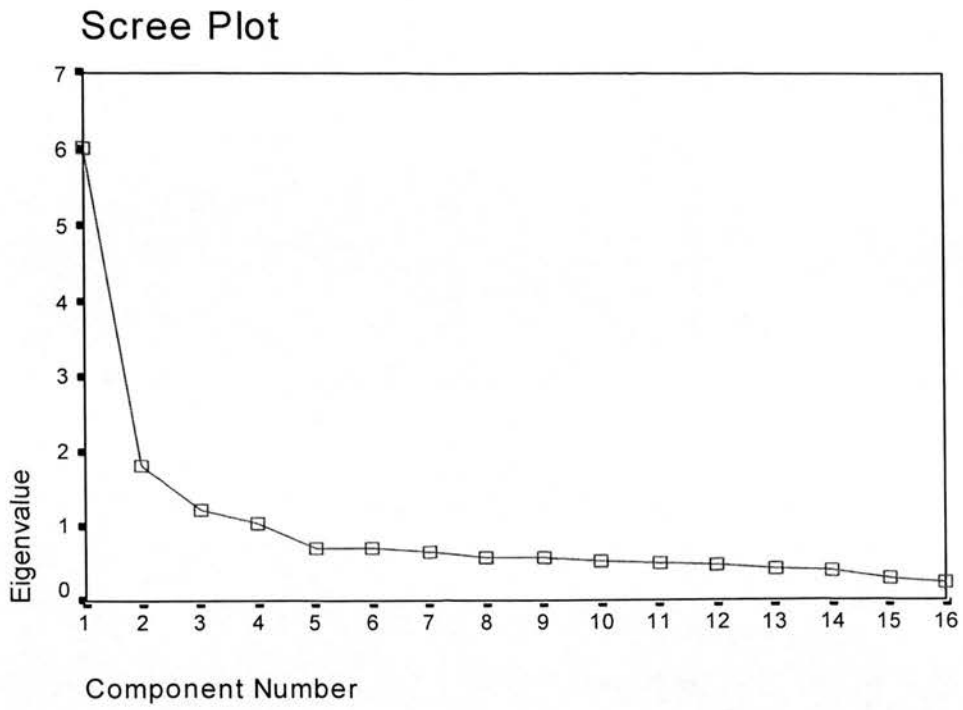


**Figure 7.1**. 'Scree slope' plot of eigenvalues from principal components analysis (unrotated factors). Factors 1-4 have eigenvalues>1, and are outliers from the shallow gradient created by factors 5-16.

### 7.3.3 Sub-group factor analyses

Because of the large number of participants, it was possible to repeat the PCA using sub-groups. For comparison based on hypoglycaemia awareness, the data were divided into those participants who gave a rating of 1 or 2 (normal awareness) and those who gave a rating of 3 to 7 (impaired awareness) [9]. Results of this sub-group analysis are given in Tables 7.6. Although factor ordering is different, essentially the same symptoms loaded onto the same four factors as in the analysis of the full data set.

The same process was repeated to compare subjects classified according to glycaemic control (HbA1c above and below the median) and age (above and below the median). Neither lower *vs.* higher HbA1c, nor lower *vs.* higher age, modified the PCA results (data not shown).

| Symptom | Normal awareness Rotated factor loadings | | | | Impaired awareness Rotated factor loadings | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| **Thirst** | **.88** | -.01 | .15 | -.13 | -.01 | **.89** | -.04 | .00 |
| **Dry mouth** | **.82** | .01 | .03 | .02 | .09 | **.82** | .15 | .10 |
| **Not feeling right** | **.57** | .06 | -.26 | .03 | .36 | .12 | .28 | -.23 |
| **Need to urinate** | **.83** | -.12 | .09 | .01 | -.10 | **.68** | -.01 | -.19 |
| **Tiredness** | .23 | .29 | -.27 | -.23 | **.43** | .10 | -.04 | **-.37** |
| **Irritability** | .05 | .01 | **-.76** | -.01 | .05 | .04 | .10 | **-.78** |
| **Poor concentration** | .16 | .34 | **-.46** | -.26 | **.42** | -.06 | .04 | **-.44** |
| **Weakness** | .30 | .28 | -.22 | **-.55** | **.77** | .25 | -.07 | .10 |
| **Sweet/odd taste** | **.59** | -.04 | -.25 | .23 | .27 | .18 | **.62** | .21 |
| **Restlessness** | .04 | -.07 | **-.80** | .15 | .02 | .09 | .07 | **-.76** |
| **Feeling tense** | -.16 | -.06 | **-.87** | -.05 | .07 | .03 | -.03 | **-.81** |
| **Headache** | .15 | .32 | -.21 | **.55** | -.01 | -.04 | **.83** | -.02 |
| **Blurred vision** | -.09 | **.79** | .09 | -.03 | **.70** | -.07 | -.00 | .00 |
| **Light headedness** | -.03 | **.72** | -.14 | .08 | **.78** | -.08 | .09 | -.14 |
| **Nausea** | .18 | **.50** | -.03 | **.42** | -.17 | -.00 | **.79** | -.16 |
| **Dizziness** | -.04 | **.86** | .10 | -.02 | **.75** | -.14 | .04 | -.22 |

**Table 7.6.** Results of principal components analysis in subjects with normal and impaired hypoglycaemia awareness.

### 7.3.4 Factors associated with symptom intensity and glycaemic threshold

A mean symptom intensity score was calculated for each participant as the mean of scores for the 16 symptoms identified in section 7.3.1. A multiple regression model was constructed, with mean symptom intensity as the dependent variable, and the following as independent variables: age, duration of insulin therapy, hypoglycaemia awareness rating, current $HbA_{1c}$, sex, type of diabetes, and use of beta-adrenoceptor blocking drugs. Non-significant independent variables were removed in stepwise fashion, with a threshold for significance of P=0.05. Results are given in Table 7.7. There was a significant negative association with age, and a statistically significant but small positive association with $HbA_{1c}$; the total $R^2$ for this model was 0.197.

A total of 340 subjects (85%) were able to estimate a glycaemic threshold for the onset of symptoms of hyperglycaemia. The mean (range) estimated threshold was 15.1 (8–30) mmol/L. A multiple regression model with estimated glycaemic threshold as the

dependent variable and the same independent variables as before was constructed. There were statistically significant but small associations with hypoglycaemia awareness, age, $HbA_{1c}$ and sex; however, the variance explained by this model was small (total $R^2$ 0.118).

| Dependent variable | Independent variable | $R^2$ change (total $R^2$) | Standardized beta | P |
|---|---|---|---|---|
| **Mean symptom intensity** | Age | 0.182 | -0.410 | <.001 |
| | $HbA_{1c}$ | 0.015 | 0.125 | .007 |
| | | (0.197) | | |
| **Blood glucose threshold for onset of symptoms** | Hypo awareness | 0.062 | 0.259 | <.001 |
| | Age | 0.020 | 0.160 | .003 |
| | $HbA_{1c}$ | 0.018 | 0.154 | .005 |
| | Sex | 0.018 | -0.137 | .012 |
| | | (0.118) | | |

**Table 7.7**. Results of multiple regression analyses, with (i) mean symptom intensity and (ii) estimated glycaemic threshold for symptoms as the dependent variables. Only associations yielding P<.05 are shown. Total $R^2$ is provided for each model in brackets. Standardized beta coefficients are the change in the dependent variable for each standard deviation of the independent variable; they are utilized here because the absolute spread of age (18-84) and $HbA_{1c}$ (6-13) are very different. For example, for mean symptom intensity, age has a greater effect than $HbA_{1c}$ overall, but because the spread of age is much greater the increment in symptom intensity per year of age is less than the increment per % of $HbA_{1c}$.

### 7.3.5 Other symptoms of hyperglycaemia

With open-ended questioning, 68 (17%) of subjects reported hyperglycaemic symptoms other than the 18 included in the questionnaire. The commonest were sweating (15 subjects), muscular ache (14 subjects), a flushed or warm sensation (14 subjects) and heavy or stiff limbs (10 subjects). Various other symptoms were each reported by fewer than five subjects.

## 7.4 Discussion

In the present study, 90% of adults with insulin-treated diabetes reported experiencing symptoms associated with hyperglycaemia. The mean estimated blood glucose level at which hyperglycaemic symptoms develop was 15 mmol/l, though the range of estimates was wide (8 to 30 mmol/l). In regression analyses, the mean symptom intensity was modestly associated with age, with older respondents reporting less intense symptoms. The estimated glycaemic threshold was weakly associated with hypoglycaemia awareness, such that people with diminished awareness of hypoglycaemia tended to report higher blood glucose levels for the onset of hyperglycaemic symptoms. This association may be explained by tolerance to both hypo- and hyperglycaemic extremes in individuals with erratic blood glucose control, or by a defect in glucose sensing leading to a single state of impaired glycaemic awareness.

When symptoms were examined using principal components analysis, most of the co-variation between symptom intensity scores could be accounted for by four factors. Based on the symptoms associated with each factor, they have been labelled here as *osmotic, neurological, mental agitation* and *malaise.* The osmotic symptoms were the most easily classifiable and strongly endorsed symptoms, and are the classically described symptoms of hyperglycaemia. The 'mental agitation' and 'neurological' symptoms together suggest some change in cerebral function due to hyperglycaemia, and there is experimental evidence to support this. The 'neurological' symptoms (light headedness, blurred vision and dizziness) raise the possibility of acute hyperglycaemia-induced hypotension. To our knowledge, this has not been described; on the contrary, an acute rise in blood pressure during hyperglycaemia is well described [18]. However, there is a potential pitfall in the interpretation of factor analysis of symptom scores. In previous papers on the symptoms of hypoglycaemia, factor analysis was valuable in suggesting separate underlying physiological processes (neuroglycopenic and autonomic nervous) [2,3,4], symptoms correlating because they are different manifestations of the same physiological aspect of hypoglycaemia. This holds true for thirst and polyuria in the present study, which are clearly separate symptoms attributable to the osmotic effect of hyperglycaemia. However, symptom scores may also correlate because they are different verbal descriptions of the same sensation. The 'neurological' symptoms could

be linguistically associated in this way, rather than because they represent another physiological process (such as hypotension).

The caveat above notwithstanding, the factor analysis results do strongly indicate that hyperglycaemia is associated with effects on cerebral function as well as physical symptoms. There is experimental evidence to support this. Sommerfield *et al* compared the effects of euglycaemia (4.5 mmol/l) and hyperglycaemia (16.5 mmol/l) in subjects with type 2 diabetes [13]. During hyperglycaemia, subjects performed significantly less well on tasks measuring information processing (choice reaction time, Trail Making B and the Digit Symbol Substitution Test), working memory and attention. Mood was also affected during hyperglycaemia, with questionnaire responses indicating reduced happiness and alertness and increased tension. Physiological mechanisms for impaired cognition during hyperglycaemia are not obvious, but may relate to cerebral fluid or electrolyte shift. Distraction, or loss of attention, caused by the physical symptoms may also contribute.

Hypoglycaemia-induced cognitive dysfunction has been an important area of clinical diabetes research. Could hyperglycaemia-induced cognitive dysfunction also be important? The data of Sommerfield *et al* confirm its existence, although the magnitude of impairment at 16.5 mmol/l was considerably less than has been seen at 2.5 mmol/l. Sommerfield *et al* recorded 5 to 7% slower completion of Trail Making and Digit Symbol Substitution tasks, whereas completion was 10 to 20% slower in the study described in Chapter 6. However, greater cognitive impairment might be seen at higher blood glucose concentrations, e.g. 20 mmol/l or higher. While at first such hyperglycaemia may seem extreme and unrepresentative, it is probably a common occurrence. A person with diabetes achieving an $HbA_{1c}$ of 8.5% (about average for type 1 diabetes in the Lothian region of Scotland) has a mean blood glucose of approximately 11 mmol/l [19]. In such a person, hypoglycaemia at 2.5 mmol/l represents an excursion from the mean of 8.5 mmol/l; the hyperglycaemic equivalent is 19.5 mmol/l, but as the distribution is positively skewed, hyperglycaemic excursions will tend to be more extreme than hypoglycaemic excursions. It would be interesting to repeat the study of Sommerfield *et al* at a higher blood glucose level, provided safety could be assured and research ethics requirements satisfied.

Another issue affecting the clinical relevance of hyperglycaemia-associated cognitive dysfunction is the possible adaptation of symptoms and cognition to repeated episodes of hyperglycaemia. As discussed in section 2.5, people exposed to recurrent hypoglycaemia seem to show cognitive adaptation, but this is insufficient to compensate for the loss of symptoms. Our clinical experience strongly suggests that people with poorly-controlled diabetes develop tolerance to the hyperglycaemic state, feeling no symptoms at blood glucose concentrations as high as 15-20 mmol/l, but developing symptoms of hypoglycaemia at 7-10 mmol/l. There was little evidence for this in the present study, with only a trivial association between $HbA_{1c}$ and estimated symptom threshold. Such retrospective estimates may be unreliable, particularly as people with poorly-controlled diabetes are less likely to measure blood glucose frequently. It would be interesting to repeat studies of hyperglycaemic cognitive function in people with well- and poorly-controlled diabetes, although obtaining statistical power for between-groups analyses (discussed in Chapters 5 and 8) would be problematic.

People with diabetes may use symptoms to identify periods of hypo-and hyperglycaemia. This study indicates that there are some similarities between the two, with common symptoms including tiredness, light-headedness, restlessness, poor concentration, headache and nausea. Symptoms of thirst, dry mouth and need to urinate have not been associated with hypoglycaemia, and these symptoms appear to be specific for hyperglycaemia. The autonomic symptoms of hypoglycaemia (sweating, palpitations, shaking and hunger) were not reported as symptoms in the present study. It would appear, therefore, that the two states may be best distinguished by the presence of osmotic or autonomic symptoms, whereas a variety of mental and physical symptoms may be common to both.

It was noted incidentally that many subjects reported that symptoms of tiredness and aching muscles were more common during early hyperglycaemia, whereas thirst and increased urination emerged with more prolonged or severe hyperglycaemia. It is plausible that thirst and polyuria would be delayed, as they presumably cannot develop until there has been a sustained diuresis. Participants were not formally asked about symptoms at different levels or durations of hyperglycaemia, and so the study design did

not permit assessment of this putative association. However, an absence of osmotic symptoms in early hyperglycaemia would be expected to make identification of this state more difficult. The ability to distinguish hypo- and hyperglycaemia could be further impaired in people whose autonomic symptoms of hypoglycaemia are diminished in association with long duration of diabetes or frequent exposure to hypoglycaemia [1]. Previous studies have confirmed that estimation of blood glucose from symptoms is unreliable in many patients [12,20,21].

The symptoms of hyperglycaemia identified in this study were similar to those reported in other studies [12,14,16]. Some subjects also reported symptoms of hyperglycaemia other than those included in the questionnaire, most commonly sweating, myalgia, a flushed or warm sensation, and heavy or stiff limbs. These symptoms were reported rarely, although it is possible that more frequent endorsement would have resulted from prompted questions. Future studies should include these symptoms.

Further research in this area should include studies to determine the threshold for hyperglycaemic symptoms under experimental conditions, with comparisons between diabetic people with normal and impaired hypoglycaemia awareness, and strict and poor glycaemic control. It would also be pertinent to determine: how soon symptoms develop after the onset of hyperglycaemia; whether the duration and degree of hyperglycaemia affect the nature and correlational structure of the symptoms experienced; and whether the four groups of symptoms identified in the present study are differentially affected.

## 7.5 References


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# Chapter 8

# Discussion and Conclusions

## 8.1 Discussion and Conclusions

### 8.1.1 The effects of hypoglycaemia on memory consolidation, and fMRI brain activation during recall (Chapter 4)

In this study, an hour of moderate hypoglycaemia (2.5 mmol/l) between the learning and recall phases of a memory task appeared to have no effect on the consolidation of visual stimuli in non-diabetic volunteers. There also appeared to be no effect of hypoglycaemia on brain activation during the testing of recognition.

Strictly speaking, the appropriate conclusion is that no significant effect at $P<0.05$ was seen in this study, which had 80% power to detect an effect of 0.75 SD. In the study reported in Chapter 5 (which was carried out later, and could not inform the sample size of this study), the greatest effect of hypoglycaemia was seen for delayed verbal memory performance, with an effect equivalent to approximately 0.6 SD. *Post hoc* calculation reveals that for 80% power to detect a similar effect on the word task in the present study, a sample size of 31 would have been needed. Jauch-Chara *et al* recently published a study of the effects of hypoglycaemia on the overnight consolidation of memory [1]. The decline in memory performance associated with hypoglycaemia in their study was approximately 0.35 SD, and using this estimate of effect size, approximately 50-80 subjects would have been needed to achieve 80% power in the present study. With hindsight, the study was underpowered to detect an effect of hypoglycaemia on consolidation.

On the other hand, in the present study, memory performance scores following hypoglycaemia and euglycaemia were identical, which does not support the possibility of

an effect of hypoglycaemia failing to reach significance. If one discounts issues of statistical power and accepts the present study as clearly negative, then the most likely explanation for the significant results obtained by Jauch-Chara *et al* is that they induced hypoglycaemia during sleep. Sleep is well-established as a time of active re-processing of memory [2], and may also be a time of increased susceptibility to hypoglycaemia.

More profound hypoglycaemia within the design of the present study (e.g. 1.5-2.0 mmol/L) might yield a significant effect on memory consolidation, and this would be interesting to do if this degree of hypoglycaemia can be achieved safely.

In conclusion, this study provides some reassurance that moderate hypoglycaemia, which occurs frequently in the daily lives of people with insulin-treated diabetes, does not disrupt memory of prior events. It would be valuable to repeat the sleep study of Jauch-Chara *et al* for confirmation of their findings, and if possible to include functional brain imaging during the testing phase to reassess the possibility of hippocampal dysfunction following hypoglycaemia. It may also be interesting to repeat the design of the present study but with more profound hypoglycaemia.

### 8.1.2 The effects of acute hypoglycaemia on learning, recall and prospective memory (Chapter 5)

There were two main findings in this study. Firstly, moderate hypoglycaemia (2.5 mmol/l) was associated with poorer memory performance, irrespective of whether it occurred during the learning phase or the recall (testing) phase. For a student with diabetes, this indicates that hypoglycaemia would be disadvantageous whether occurring during revision or an examination, with similar consequences in other settings. Secondly, the novel prospective memory task showed a statistically-significant deterioration with hypoglycaemia.

The prospective memory results are important, because the task mimics one of the true roles of memory in daily life – remembering to do something. As briefly discussed in section 2.2.2, the practical relevance of many cognitive function tests is unclear. One of

the most widely-used memory tests is the Rey Auditory Verbal Learning Test (AVLT), also used in the present study, which tests subjects for their ability to recite back a list of 15 words. This mode of learning may be seen in primary schools, and may indeed correlate well with 'general' memory performance, but its direct relevance to an adult with diabetes is limited. Another common test is paired-associates, as used by Jauch-Chara *et al* [1], in which the subject is first read pairs of nouns, and later required to recall the second word when the first of the pair is read. It is hard to think of any activity in daily life that resembles this task. The present study is believed to be the first hypoglycaemia study to utilize a test specifically mimicking daily life. Although it was not trialled in the field, the authors of the paper on which it was based did do this, obtaining a good correlation between performance on the laboratory-based video task and performance on the same task in the streets of Dunedin, New Zealand [3].

The magnitude of the impairment associated with hypoglycaemia must be considered when extrapolating to daily life. The performance reduction in this study was approximately 12%, or somewhere between a 'small' and 'medium' effect by Cohen's d statistic [4]. This effect size is similar to those seen for various cognitive tests in Chapter 6, and in numerous other studies from this centre [5,6,7,8,9]. It is important that clinical advice and guidelines for people with diabetes should not be dogmatic: in many cases, hypoglycaemia may cause only a minor impairment, rather than rendering them incapable, and the degree of impairment is likely to be related to the level of hypoglycaemia. By contrast, in the studies by Sommerfield *et al* from this centre [10,11], the reductions in memory performance were very much greater – in many cases around 50% impairment, with Cohen's d well above the level deemed a 'large' effect. In the studies of Sommerfield *et al,* both learning and recall took place during the experimental condition. It would be valuable to repeat the prospective memory task, but with hypoglycaemia occurring across both learning and recall, to assess the maximum impact of moderate hypoglycaemia on prospective memory.

In conclusion, this study has confirmed that both the learning and recall aspects of memory are impaired by moderate hypoglycaemia, and the prospective memory task has lent authority to extrapolation of laboratory experimental results to hypoglycaemia in

diabetes. The prospective memory task is more time-consuming and requires additional equipment, but if these are surmountable then it may be preferable to use the same task or a similar task in future hypoglycaemia-memory research. With reference to the conclusions of Chapter 4, a particularly interesting study design would use this task to re-assess the effects of hypoglycaemia during sleep on memory consolidation.

### 8.1.3 The Effects of Acute Hypoglycaemia on Non-verbal Intelligence, and Accuracy and Speed of Visuospatial Cognitive Function (Chapter 6)

Non-verbal intelligence (principally mathematical and visuospatial problem solving, as assessed by the Raven's Progressive Matrices test) was not significantly impaired during hypoglycaemia in earlier studies by McAulay *et al* [6,7]. This was unexpected, but may have been due to a ceiling effect, resulting from the use of a moderately difficult test in a very able group of subjects. In the present study, performance on the harder version of the same test (Raven's Advanced Progressive Matrices) was impaired during hypoglycaemia at 2.5 mmol/l with clear statistical significance. Performance on the Alice Heim 5 test also deteriorated, but failed to achieve statistical significance ($P=0.057$); this may well have been due to an inadequate sample size. It may be concluded that higher-level intelligence is affected by moderate hypoglycaemia, consistent with the previous research and theories outlined in Section 2.2.4.

This study also included a maze task, intended to examine whether hypoglycaemia is more likely to affect speed or accuracy of cognition. Disappointingly, there was absolutely no effect of hypoglycaemia on either performance measure, and all that may be concluded is that the maze task is not a good test for this purpose. The unanswered question of speed *vs.* accuracy is of considerable practical relevance, for example in relation to driving.

### 8.1.4 Symptoms of Hyperglycaemia (Chapter 7)

This study has defined the symptoms of hyperglycaemia using a very large patient

population. The study confirmed that the osmotic symptoms of thirst, polyuria and dry mouth are the most commonly experienced symptoms of hyperglycaemia, and the autonomic symptoms of hypoglycaemia are not reported. Thus, the presence of one or other set of symptoms is useful in differentiating the two conditions.

One of the other groups of hyperglycaemia symptoms comprised feeling tense, restlessness, irritability and poor concentration. Although a label of 'mental agitation' was applied to this group, the symptom of impaired concentration also indicates that subjects had noticed some cognitive difficulty associated with hyperglycaemia, and the other symptoms are likely to cause difficulties with attention. This association of hyperglycaemia with cognitive dysfunction is supported by an experimental study performed subsequently in this centre by Sommerfield *et al*, in which reaction time, working memory and aspects of attention deteriorated during a hyperglycaemic clamp at 16.5 mmol/l [12].

The average estimated threshold for detection of symptoms was 15.1 mmol/l. Retrospective estimates may be inaccurate, and the mean conceals a wide range of individual responses (8 to 30 mmol/l), but even so, this tends to confirm that reliance on symptoms is inadequate for detection of hyperglycaemia in clinical practice. In multivariate analysis, age appeared to be the most important factor affecting the perception of hyperglycaemia symptoms. Older age was associated with a reduction in symptom intensity ratings that is likely to be clinically significant as well as statistically significant. The symptoms of hypoglycaemia have also been reported to be diminished in intensity in older people [13]. Together, these findings show that elderly people with diabetes are vulnerable to extremes of hypo- and hyperglycaemia because of reduced ability to detect these changes, as well as potentially reduced ability to manage them appropriately.

### 8.1.5 Statistical issues

A common theme in the studies described in Chapters 4, 5 and 6 is statistical power and sample size calculations. This was also a criticism of some previous studies in Chapter

2.

Appropriate power and sample-size calculations are crucial for quantitative biological research, because of the natural variability of almost any parameter that might be measured. It is unethical to ask subjects to submit to invasive or unpleasant procedures if the study being performed is unlikely to detect a genuine effect. In the research literature, 'negative' studies are very often described as providing evidence of no effect when in fact they show no evidence either way.

There are at least two reasons why research is sometimes deficient in this regard. Firstly, power calculations are more complex than the statistical tests to which they apply, and may be daunting for non-statisticians. Secondly, to be performed accurately, information may be required that is not available prior to the study's completion. In this case, estimates based on previous similar studies may be used instead, but the relevant data are not always published. This is particularly true for repeated-measures designs, where an essential parameter is the standard deviation of the difference between repeated measurements; this information has never reported in published hypoglycaemia studies.

The Appendix provides the data that would be required for power and sample size calculations for all tests used in the studies described in this thesis.

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# Appendix 1

# Information For Statistical Power Calculations

To assist with future studies, information regarding the within-patient variation is provided for the cognitive function tests used in the studies described in this thesis.

In each case, the difference between hypoglycaemia and euglycaemia performance scores is calculated for each patient. The mean of these differences is necessarily the same as the difference between mean hypoglycaemia and euglycaemia scores. The standard deviation is an index of the consistency of the effect of hypoglycaemia between subjects/studies, and is needed for power calculations and sample sizes.

## Trail Making B

| Study | Subjects | Completion time (sec) - euglycaemia | Completion time (sec) - hypoglycaemia | Mean diff | SD diff |
|---|---|---|---|---|---|
| Chapter 4 | Non-DM | 38.7 | 46.3 | 8.7 | 14.2 |
| Chapter 5 | T1DM, NHA | 38.3 | 46.3 | 8.0 | 14.0 |
| Chapter 5 | T1DM, IHA | 44.0 | 53.6 | 7.4 | 20.7 |

## Digit Symbol Substitution

| Study | Subjects | No. correct in 2 minutes - euglycaemia | No. correct in 2 minutes - hypoglycaemia | Mean diff | SD diff |
|---|---|---|---|---|---|
| Chapter 4 | Non-DM | 87.7 | 79.4 | 8.9 | 11.5 |
| Chapter 5 | T1DM, NHA | 79.8 | 65.6 | 14.1 | 11.3 |
| Chapter 5 | T1DM, IHA | 66.6 | 62.1 | 4.5 | 16.2 |

## Choice Reaction Time

| Study | Subjects | Mean reaction time (ms) - euglycaemia | Mean reaction time (ms) - hypoglycaemia | Mean diff | SD diff |
|---|---|---|---|---|---|
| Chapter 5 | T1DM, NHA | 612 | 666 | 54 | 57 |
| Chapter 5 | T1DM, IHA | 647 | 679 | 32 | 68 |

## Edinburgh Hypoglycaemia Scale Symptoms

| Study | Subjects | Total score - euglycaemia | Total score - hypoglycaemia | Mean diff | SD diff |
|---|---|---|---|---|---|
| Chapter 4 | Non-DM | 25.8 | 35.6 | 9.8 | 12.8 |
| Chapter 5 | T1DM, NHA | 25.0 | 35.0 | 10 | 14.8 |
| Chapter 5 | T1DM, IHA | 21.6 | 25.9 | 5.1 | 6.6 |

## Faces Recognition Task

| Study | Subjects | Total score - euglycaemia | Total score - hypoglycaemia | Mean diff | SD diff |
|---|---|---|---|---|---|
| Chapter 4 | Non-DM | 67.2 | 66.9 | 0.3 | 8.8 |

## Words Recognition Task

| Study | Subjects | Total score - euglycaemia | Total score - hypoglycaemia | Mean diff | SD diff |
|---|---|---|---|---|---|
| Chapter 4 | Non-DM | 78.0 | 77.1 | 0.9 | 9.2 |

## Rey Auditory Verbal Learning Test

| Study | Subjects | No. correct - euglycaemia | No. correct - hypoglycaemia | Mean diff | SD diff |
|---|---|---|---|---|---|
| Chapter 5 | T1DM, NHA | 10.0 | 8.7 | 1.3 | 2.6 |
| Chapter 5 | T1DM, IHA | 10.9 | 9.2 | 1.6 | 2.7 |

*NB – in Chapter 5, only the learning phase of the AVLT was performed during hypoglycaemia/ euglycaemia. Recall was always tested during euglycaemia.*

## Wechsler Logical Memory Test

| Study | Subjects | No. correct - euglycaemia | No. correct - hypoglycaemia | Mean diff | SD diff |
|---|---|---|---|---|---|
| Chapter 5 | T1DM, NHA | 13.1 | 11.0 | 2.1 | 3.2 |
| Chapter 5 | T1DM, IHA | 15.7 | 13.7 | 2.0 | 4.1 |

*NB – in Chapter 5, only the learning phase of the Logical Memory Test was performed during hypoglycaemia / euglycaemia. Recall was always tested during euglycaemia.*

## Wechsler Visual Memory Test

| Study | Subjects | No. correct - euglycaemia | No. correct - hypoglycaemia | Mean diff | SD diff |
|---|---|---|---|---|---|
| Chapter 5 | T1DM, NHA | 82.8 | 77.5 | 5.3 | 16.1 |
| Chapter 5 | T1DM, IHA | 81.3 | 85.1 | 3.7 | 14.4 |

*NB – in Chapter 5, only the learning phase of the Visual Memory Test was performed during hypoglycaemia / euglycaemia. Recall was always tested during euglycaemia.*

## Prospective Memory Test

| Study | Subjects | No. correct - euglycaemia | No. correct - hypoglycaemia | Mean diff | SD diff |
|---|---|---|---|---|---|
| Chapter 5 | T1DM, NHA | 11.4 | 10.1 | 1.3 | 3.7 |
| Chapter 5 | T1DM, IHA | 15.4 | 13.3 | 2.1 | 2.9 |

*NB – in Chapter 5, only the recall phase of the Prospective Memory Test was performed during hypoglycaemia / euglycaemia. Learning always occurred during euglycaemia.*

# Appendix 2

## Published Articles

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DIABETES/METABOLISM RESEARCH AND REVIEWS
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**RESEARCH ARTICLE**

# Moderate hypoglycaemia after learning does not affect memory consolidation and brain activation during recognition in non-diabetic adults

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## Abstract

**Introduction** Some aspects of memory performance are impaired during acute hypoglycaemia. The hippocampus is critical to formation of long-term memory, and may be particularly sensitive to hypoglycaemia. This study examined whether moderate hypoglycaemia occurring after learning would disrupt the consolidation process, and used functional magnetic resonance imaging (fMRI) to identify accompanying changes in brain activation.

**Methods** Sixteen non-diabetic subjects each underwent two glucose clamp studies. During euglycaemia (4.5 mmol/L), subjects tried to memorize a series of words and a series of pictures of faces. Then, either hypoglycaemia (2.5 mmol/L) was induced for one hour, or euglycaemia was maintained. During subsequent uncontrolled euglycaemia, subjects' recognition of the word and face stimuli was tested, with simultaneous fMRI to measure brain activation during recognition.

**Results** Face identification scores were 67.2% after euglycaemia and 66.9% after hypoglycaemia ($p = 0.895$). Word identification scores were 78.0 and 77.1% respectively ($p = 0.701$). Analysis of the fMRI identified two foci where activation was altered after hypoglycaemia compared with euglycaemia, but these were not in regions associated with memory, and were probably statistical artefacts.

**Conclusions** One hour of hypoglycaemia at 2.5 mmol/L induced 20–40 min after learning did not disrupt memory consolidation. fMRI did not show evidence of altered brain activation after hypoglycaemia. Consolidation may be relatively resistant to hypoglycaemia, or may have been complete before hypoglycaemia was induced. The study was powered to detect a large effect, and provides some reassurance that moderate hypoglycaemia does not cause major disruption of previously learned memories in people with insulin-treated diabetes. Copyright © 2007 John Wiley & Sons, Ltd.

**Keywords** diabetes; hypoglycaemia; memory; consolidation; functional imaging

## Introduction

Cognitive abilities, ranging from simple motor tasks and basic sensory processing to complex reasoning, are adversely affected by hypoglycaemia [1]. We have demonstrated previously that episodic short-, working- and long-term memory are significantly impaired by hypoglycaemia [2–4]. These

results are in agreement with earlier reports, which included some tests of memory among broader cognitive test batteries, but much remains to be elucidated about the functioning of memory during hypoglycaemia.

Memory has been conceptualized as composed of three main subsystems: sensory memory, short-term memory, and long-term memory [5]. Sensory memory is the brief retention of an auditory or visual impression after the stimulus has been removed. Short-term memory can hold a limited number of items, with a duration measured in seconds. Long-term memory is durable, with no quantifiable capacity limits. The conversion of short- to long-term memory is known as consolidation. The neurological basis of consolidation is not well understood, but the medial temporal lobe structures appear to be critical. Evidence comes from individuals who have sustained focal damage to the hippocampus and have developed near-total inability to form new long-term memories, while retaining intact short-term memory and pre-formed long-term memories [6–9]. Cases have been reported of individuals who have developed amnesia following severe hypoglycaemia, in whom frontal and hippocampal lesions were identified on magnetic resonance imaging (MRI) scans [10,11]. A similar distribution of lesions was reported on MRI scans in four patients in a vegetative state following severe hypoglycaemia [12], and from post-mortem examinations following fatal hypoglycaemia in a human case report [13], and in an experimental rat study [14]. The frontal lobes and hippocampal region appear to be particularly sensitive to hypoglycaemia, and this may contribute to the disruptive effects of moderate hypoglycaemia on aspects of memory.

In most previous studies of memory during hypoglycaemia, both stimulus presentation and recall testing took place during the experimental condition (hypoglycaemia or euglycaemia), and so these studies could not distinguish the effects of hypoglycaemia on acquisition (learning), recall, and consolidation. We have recently demonstrated that moderate hypoglycaemia impairs both learning and recall [15]. If hypoglycaemia also impairs consolidation, then depending on the duration of the consolidation process, it could impair recall of information learned prior to the onset of hypoglycaemia.

The present study investigated whether a period of hypoglycaemia occurring immediately after exposure to new stimuli impaired subsequent recognition. Functional magnetic resonance imaging (fMRI) was used to examine brain activation during the recognition task. It was hypothesized that activation in the hippocampus and adjacent medial temporal lobe structures would be reduced following hypoglycaemia. fMRI exploits a natural difference in the magnetic properties of oxy-haemoglobin and deoxy-haemoglobin [16], such that the blood-oxygen level dependent signal is increased following neuronal activation [17,18]. Previous fMRI studies have shown that activation of the medial temporal lobe structures (including the hippocampus) is consistently associated with successful encoding and retrieval of episodic memories [19–23].

## Methods

The study was approved by the local research ethics committee, and all subjects provided informed consent.

### Subjects

Sixteen right-handed non-diabetic adults (9 male) for whom English was a first language were recruited. People with active medical conditions or previous history of seizure, cerebral injury, or other contra-indication to experimental hypoglycaemia were excluded. Median age was 25 years (range 20–44) and median body mass index was 22.6 kg $m^{-2}$ (range 20.1–27.7).

### Study outline and glucose clamp procedure

Subjects underwent two glucose clamp studies (hypoglycaemia and euglycaemia), preceded by overnight fasting, and separated by at least two weeks. Soluble human insulin was infused intravenously at a fixed rate (60 mU/min/$m^2$ body surface area), and 20% dextrose was infused at a variable rate to achieve target blood-glucose concentrations. Samples for measurement of whole blood glucose were drawn every 3–5 min from a vein in the non-dominant hand, which was placed within a heated blanket to arterialize venous blood.

The study design is illustrated in Figure 1. During an initial euglycaemic phase, blood-glucose concentrations were stabilized at 4.5 mmol/L for 30 min, and the series of faces and words were presented for learning. In hypoglycaemia studies, the glucose infusion rate was immediately reduced to lower blood glucose quickly to a target of 2.5 mmol/L (deemed to be achieved when measurements over at least 6 min were in the range 2.3–2.7 mmol/L), maintained at this level for 60 min, and finally raised to 4.5 mmol/L, when the clamp was discontinued. In euglycaemia studies, blood glucose was maintained at 4.5 mmol/L for 100 min, including 40 min to match the time taken to achieve and reverse hypoglycaemia.

After discontinuation of the clamp, subjects were given a meal and allowed to rest for 1 h to ensure recovery of

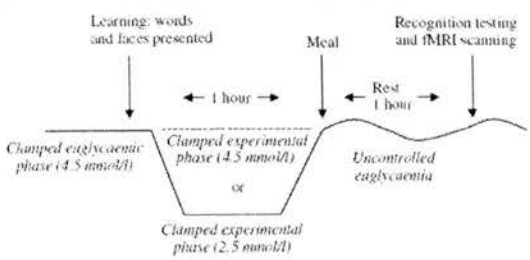


**Figure 1. Sequence of experimental procedures**

cognitive function. Subjects were then taken to the MRI suite for recognition testing and fMRI acquisition.

### Memory tests, procedure and analysis

Face stimuli were digitized photographs of a Caucasian student population, taken without jewellery or spectacles, and scaled so that the head occupied 80% of the frame. Word lists were made up of words with frequencies between 20 and 30 in the series from Kucera & Francis [24]. Two learning batteries of 40 faces (20 male, 20 female) and 50 words were created. Tests in separate subjects prior to the commencement of this study yielded recognition rates of approximately 70% after an interval of 2 h.

A different learning battery was presented at each subject's two study sessions. Stimuli were presented on a computer screen positioned at arms length. To improve retention, subjects were asked to rate each word or face for its attractiveness, although these data (which are not relevant to the current study) were not analysed. During the next hour subjects completed tests of fluid intelligence (Raven's Advanced Progressive Matrices and the Alice Heim V test), to prevent rehearsal of learned material, and to address a separate research question; results of these have been published elsewhere [25].

Recognition was tested when subjects were in the MRI scanner. Subjects viewed stimuli on a screen directly above their faces, with focal aids if required. Stimuli were presented using E-prime Version 1.0 (Psychology Software Tools Inc, Pittsburgh, USA) and IFIS 1.09 (MRI Devices Corporation, Waukesha, USA). Following an initial practice run, subjects viewed 90 faces, of which 40 had been seen before (targets) and 50 were new (decoys). Subjects identified targets and decoys by pressing left- and right-hand buttons. Word recognition was tested with 140 words, comprising 50 targets and 90 decoys.

Performance was analysed using repeated-measures analysis of variance. Order of glycaemic condition and order of stimulus battery were counterbalanced within the experimental design, and were included as between-subjects factors. Analyses were performed using SPSS 11.0 (SPSS Inc, Chicago, USA).

### Imaging acquisition and analysis

Scanning was carried out on a 1.5T GE Signa scanner fitted with Echospeed gradients, using the standard head coil. Acquisition periods were 540 (face paradigm) and 840 s (word paradigm); the first 10 s were discarded to ensure steady-state magnetization. Contiguous gradient echo, echoplanar images (TR 2500 ms, TE 40 ms) were collected from 30 5mm slices (interleaved acquisition) parallel to the anterior–posterior commissure plane. The matrix acquired was 64 × 64 with an in-plane resolution of 3 × 3 mm. Button responses to the memory tasks were logged simultaneously. A T1-weighted structural scan was acquired after fMRI acquisition. Images in each series were registered to the initial image to correct for head motion. EPI-volumes were then co-registered to the T1-weighted structural volume and aligned to standard coordinates, and data was smoothed with a 6 mm Gaussian kernel to allow for variability in gyral anatomy and location of activation between subjects.

The fMRI design for the test was event-related. Data were analysed using the statistical parametric mapping package SPM99. Events were modelled using canonical haemodynamic response functions and six movement regressors. Events were classified by subject responses as 'Correct Hit', 'Correct Rejection, 'Incorrect Hit', or 'Incorrect Rejection'. For a small number of events no response was given, and these were assigned to the relevant incorrect category. First-level fixed-effects T-contrasts were made between the euglycaemia and hypoglycaemia conditions. The T threshold was set at 3.09, and cluster size threshold at 10 voxels. A fixed-effects analysis was performed, with a view to further analysis if this produced promising results.

## Results

### Blood glucose and timing

Comparisons for hypoglycaemic (H) and euglycaemic (E) sessions were as follows. Mean (SD) blood glucose during the initial euglycaemic phase was 4.4 (0.2) mmol/L (H) *versus* 4.3 (0.2) mmol/L (E), $p = 0.436$. Mean (SD) blood glucose during the experimental phase was 2.5 (0.2) mmol/L (H) *versus* 4.4 (0.3) mmol/L (E). In hypoglycaemia sessions, mean (SD) time to achieve stable hypoglycaemia was 30 (7) min. Mean (SD) interval between discontinuation of clamp and commencement of memory testing was 86 (10) min (H) *versus* 85 (15) min (E), $p = 0.803$.

### Memory performance

Stimuli correctly identified were converted into a percentage, weighted for the true frequency of targets and decoys so that the chance score was 50%. Adjusted face identification scores were almost identical following euglycaemia and hypoglycaemia [67.2 (8.2) % and 66.9 (7.4) % respectively; $\eta^2 = 0.002$, $p = 0.895$], as were adjusted word identification scores [78.0 (8.0) % and 77.1 (12.2) % respectively; $\eta^2 = 0.013$, $p = 0.701$].

There was variation in the time intervals between learning and attainment of hypoglycaemia, and between learning and testing. Memory performance was re-analysed with these intervals as covariates, but all effects remained clearly non-significant. There were also no significant effects for unadjusted scores (e.g. number of correct hits). No individual memory item was very easy (correctly identified by >13 subjects) or very hard (correctly identified by <3 subjects).



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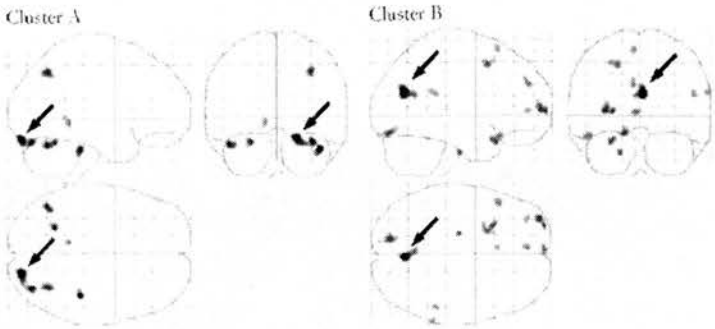


| Cluster | X | Y | Z | Voxels | P |
|---|---|---|---|---|---|
| A | 20 | -88 | -20 | 120 | 0.016 |
| B | 4 | -68 | 22 | 108 | 0.027 |

**Figure 2. Brain activation maps showing clusters achieving $p < 0.05$ for hypoglycaemia *versus* euglycaemia. Cluster A showed significantly less activation following hypoglycaemia for faces correctly identified as targets. Cluster B showed significantly more activation following hypoglycaemia for all faces**

*Post hoc* calculation indicated that the study had 80% power to detect a difference in adjusted face scores of 6.6 (0.8 euglycaemia SD), and in adjusted words scores of 6.9 (0.86 euglycaemia SD).

### Brain activation

One brain area appeared to show glycaemic differences in recognition-associated activation (Figure 2, cluster A). This cluster is located on the border of the right occipital cortex and cerebellum, and showed reduced activation after hypoglycaemia for faces correctly identified as targets. A second area (Figure 2, cluster B) in the midline close to the calcarine sulcus showed increased activation following hypoglycaemia for all faces, irrespective of response.

Neither area is known to be associated with memory. The statistical significance is borderline using the liberal fixed-effects model, and in the absence of any results requiring further exploration, a more conservative random-effects analysis was not performed.

## Discussion

No effect of hypoglycaemia on memory performance was seen in the present study. It is possible that hypoglycaemia has no effect on consolidation, but at least five alternative explanations can be proposed. In this sense, the null findings are helpful to further research.

First, the study may have been underpowered, as advance sample size estimates were limited by a lack of previous data. *Post hoc* calculations showed that the study had good power to detect a 'large' effect, according to Cohen's conventions [26]. We previously published a study of hypoglycaemia during learning and recall of verbal and visual material (this was carried out after the present study and could not inform its sample size) [15]. The greatest effect of hypoglycaemia was seen for delayed verbal memory, with a 'medium' effect equivalent to approximately 0.6 standard deviations. A *post hoc* calculation reveals that for 80% power to detect a similar effect on the word task in the present study, a sample size of 31 would have been needed. However, although not statistically rigorous, the almost identical results following euglycaemia and hypoglycaemia do not support the possibility of an effect failing to achieve significance.

Second, the level of hypoglycaemia induced (2.5 mmol/L) may have been insufficient. Although it is known that the entire memory process from learning to recall is affected by hypoglycaemia at 2.5 mmol/L [2,3], more profound hypoglycaemia may be necessary to disrupt consolidation.

Third, hypoglycaemia may have been achieved too late (20–40 min after stimulus exposure) to affect consolidation. The duration of the consolidation process is not clearly defined. Diverse interventions have been shown to affect memories acquired minutes or hours beforehand, including drug administration [27–29] and concussion [30]. There is also substantial evidence for the improving effects of sleep, immediately or overnight, on memory consolidation [31,32]. Other studies have shown evidence of consolidation over much longer time periods. Recognition-associated fMRI brain activation progressively decreased in the hippocampus and increased in the pre-frontal region when measured 1, 2, 30 and 90 days after stimulus exposure [31], and patients with focal hippocampal lesions display a temporal gradient for amnesia over years [33–35], suggesting transfer of memory from hippocampal to other sites. In summary, consolidation appears to be a chronic and heterogeneous process. There may be an early phase (e.g. less than 20 min after learning) which is susceptible to acute metabolic derangement; if so, a different result might be expected if hypoglycaemia could be induced instantaneously, but this would not be relevant to people

with diabetes, in whom hypoglycaemia may develop over hours [36].

Fourth, it is possible that the brain may work harder, or differently by recruitment of other regions, to achieve the same result during hypoglycaemia. However, the fMRI data revealed apparent differences in activation for hypoglycaemia and euglycaemia in only two brain areas, neither of which are associated with memory, and a liberal fixed-effects analysis yielded only borderline statistical significance. We conclude that the functional imaging showed no evidence of altered brain functioning following hypoglycaemia.

Fifth, the present study measured recognition rather than recall, because of logistical difficulties in obtaining anything other than binary answers during MRI scanning. Recognition may require less robust encoding, and hence suffer less disruption from hypoglycaemia. However, recognition tasks have been used effectively in fMRI memory studies [19], and a recognition task was adequate to show changes in both memory performance and brain activation following an intervention to affect consolidation [31]. The memory tasks in the present study had not been used previously, but the results suggest that they were valid: scores fell appropriately between floor (50%) and ceiling (100%) limits, with no very easy or difficult items.

Non-diabetic subjects were studied because of the difficulties of maintaining infusions in an MRI room, required for diabetic subjects to prevent unacceptable variations in blood glucose. However, most previous studies that have compared the cognitive effects of hypoglycaemia in diabetic and non-diabetic subjects have reported either similar results, or relative resistance to cognitive dysfunction in diabetic subjects [37]. It seems unlikely that a positive result would be obtained in diabetic subjects but a null result in non-diabetic subjects.

Jauch-Chara *et al.* recently reported the effects of hypoglycaemia on overnight memory consolidation [38]. Word pair recall was tested before and after sleep, on hypoglycaemia nights (nadir blood glucose 2.2 mmol/L induced 60 min after sleep onset) and control nights. Performance improved after control nights, consistent with the known effects of sleep on consolidation, but no improvement occurred over hypoglycaemia nights. The results suggest that hypoglycaemia did disrupt consolidation, and it is instructive to compare that study with the present study. First, Jauch-Chara *et al.* did not report a significant effect of hypoglycaemia in subsets of 16 diabetic and 16 non-diabetic subjects, but in all 32 subjects combined. This is consistent with the sample size estimate of 31 mentioned above, and tends to reinforce the lack of power of the present study. Second, consolidation while asleep may be a different phenomenon from consolidation awake while. Sleep appears to be a time of active re-processing of memory, and therefore, perhaps a time of increased susceptibility to memory interventions [39].

Other researchers have managed to study brain activation during hypoglycaemia. Using very long tubing to infusion pumps outside the MRI field, Rosenthal *et al.* examined the effects of hypoglycaemia (2.5 mmol/L) on fMRI brain activation during finger tapping and reaction time tasks in non-diabetic subjects [40]. By comparison with euglycaemia, during hypoglycaemia there was increased activation in the parietal association area, and decreased activation in the visual cortex, cerebellum, hippocampus, and pre-motor cortex. The hippocampus was the only one of these areas that was not activated by the cognitive tasks. Teves *et al.* used positron emitting tomography (PET) to measure cerebral blood flow (CBF) during passive hypoglycaemia (3.0 mmol/L) in non-diabetic subjects [41]. Global CBF fell by 6–8% during hypoglycaemia, but with a relative increase in CBF in the thalamus, medial pre-frontal cortex and right orbital pre-frontal cortex, and a 26% relative decrease in CBF in the hippocampus. Anderson *et al.* reported that occipital activation during visual stimulation was reduced during hypoglycaemia (2.8 mmol/L) in non-diabetic subjects [42]. Wessels *et al.* studied activation during a working memory test, and found increased activation in the anterior cingulate and orbital frontal gyrus during hypoglycaemia (~2.3 mmol/L) in subjects with advanced diabetic retinopathy compared with subjects with no retinopathy [43]. This was interpreted as an altered brain response compensating for cerebral microvascular damage, for which retinopathy is a proxy. It is impossible to amalgamate these studies to obtain a coherent account of changes in activation throughout the brain, but the hippocampus stood out as having reduced fMRI activation or CBF during hypoglycaemia in the studies by Rosenthal *et al.* and Teves *et al.*, [40,41]. Given the sensitivity of the hippocampus to hypoglycaemic damage [12–14], it seems plausible that hippocampal dysfunction during moderate hypoglycaemia contributes to memory impairment.

In summary, 1 h of hypoglycaemia at 2.5 mmol/L after stimulus exposure did not affect subsequent recognition performance, and did not alter brain activation patterns during recognition. A large effect of moderate hypoglycaemia on memory consolidation can be excluded, and thus, the study provides some limited reassurance relevant to diabetic daily life. In retrospect, the study was underpowered to detect an effect of hypoglycaemia on memory comparable to effects seen in other studies, and future studies should probably aim to recruit 30 or more subjects. It is possible that more profound hypoglycaemia would yield a different result. It would be of interest in future to compare regional brain activation responses to memory and non-memory tasks during hypoglycaemia, and to vary the delay before induction of hypoglycaemia, and the level of hypoglycaemia achieved.

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## Conflict of interest

None declared.

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ARTICLE

# The effects of acute hypoglycaemia on memory acquisition and recall and prospective memory in type 1 diabetes

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**Abstract**
*Aims/hypothesis* Global memory performance is impaired during acute hypoglycaemia. This study assessed whether moderate hypoglycaemia disrupts learning and recall in isolation, and utilised a novel test of prospective memory which may better reflect the role of memory in daily life than conventional tests.
*Subjects and methods* Thirty-six subjects with type 1 diabetes participated, 20 with normal hypoglycaemia awareness (NHA) and 16 with impaired hypoglycaemia awareness (IHA). Each underwent a hypoglycaemic clamp with target blood glucose 2.5 mmol/l. Prior to hypoglycaemia, subjects attempted to memorise instructions for a prospective memory task, and recall was assessed during hypoglycaemia. Subjects then completed the learning and immediate recall stages of three conventional memory tasks (word recall, story recall, visual recall) during hypoglycaemia. Euglycaemia was restored and delayed memory for the conventional tasks was tested. The same procedures were completed in euglycaemic control studies (blood glucose 4.5 mmol/l).
*Results* Hypoglycaemia impaired performance significantly on the prospective memory task ($p=0.004$). Hypoglycaemia also significantly impaired both immediate and delayed recall for the word and story recall tasks ($p<0.01$ in each case). There was no significant deterioration of performance on the visual memory task. The effect of hypoglycaemia did not differ significantly between subjects with NHA and IHA.
*Conclusions/interpretation* Impaired performance on the prospective memory task during hypoglycaemia demonstrates that recall is disrupted by hypoglycaemia. Impaired performance on the conventional memory tasks demonstrates that learning is also disrupted by hypoglycaemia. Results of the prospective memory task support the relevance of these findings to the everyday lives of people with diabetes.

**Keywords** Cognitive function · Hypoglycaemia · Hypoglycaemia awareness · Memory · Type 1 diabetes

**Abbreviations**

| | |
|---|---|
| AVLT | Auditory Verbal Learning Test |
| IHA | impaired hypoglycaemia awareness |
| NHA | normal hypoglycaemia awareness |

## Introduction

Hypoglycaemia is a common side effect of insulin therapy for type 1 diabetes [1]. Experimentally induced acute hypoglycaemia causes a deterioration in performance on a wide range of cognitive tasks in humans [2]. Memory is one the of the most important cognitive domains with respect to everyday function. Previous studies applied memory tests as part of a larger battery of cognitive tests during hypoglycaemia [3–8], and the variability of reported results may relate to the use of different memory tests. Methodological variation in hypoglycaemia studies has been reviewed elsewhere [9]; particularly pertinent issues include sample size and statistical power, the method of

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measurement of blood glucose, and the target level for, and the duration of, hypoglycaemia.

Neuropsychological research indicates that memory comprises a number of subsystems, including sensory memory, short-term memory and long-term memory [10]. We have studied previously the effects of hypoglycaemia on various components of memory using consistent experimental techniques and validated memory tasks. Hypoglycaemia impairs the auditory and visual processing subsystems of sensory memory, short-term memory, working memory and delayed memory in both diabetic and non-diabetic subjects [11–16]. Working memory and delayed memory are particularly susceptible to hypoglycaemia [17].

In all previous studies, both the acquisition (learning) and recall of material took place during hypoglycaemia. Deterioration in memory performance during hypoglycaemia may have resulted from impairment of learning, consolidation (transfer of information from short- to long-term memory), recall, or any combination of these. The present study was designed to investigate whether these processes are independently sensitive to hypoglycaemia by separating the acquisition and recall phases of memory tasks so that only one occurred during hypoglycaemia.

Conventional laboratory memory tests tend to involve tasks such as learning lists of words, prose passages, and geometrical shapes. Such tests may not reflect the memory demands of daily living, which require coordination of multiple cognitive processes such as planning and vigilance, and therefore may not have ecological relevance [18, 19]. Recall of retrospective events has been distinguished from prospective memory (remembering intended actions, or 'remembering to remember'), and tests of prospective memory may have greater ecological validity [20]. Titov and Knight developed a novel test of prospective memory in which subjects memorised a shopping list and then viewed a video of a pedestrian journey around an urban shopping area, reporting items to be purchased when specified shops appeared [21]. This video-based memory task had good test–retest reliability. It also had good criterion validity: it correlated well with performance on the same task when subjects actually walked through the shopping area. Comparative scores were obtained for 35 subjects, yielding a correlation of $r=0.71$, $p<0.001$, and no significant effect of study type (video vs in vivo) in ANOVA. A similar video task was created for the present study to explore the effects of hypoglycaemia on prospective memory in a practical setting.

Evidence has accumulated to show that recurrent exposure to hypoglycaemia in people with type 1 diabetes results in cerebral adaptation, such that cognitive performance is relatively preserved during hypoglycaemia [22–27]. Repeated exposure to hypoglycaemia can also result in impaired hypoglycaemia awareness, and a shared mechanism for the two phenomena has been proposed [28]. Thus, in studies of people with type 1 diabetes, it is important that subjects with impaired hypoglycaemia awareness (which may be a surrogate marker of cerebral adaptation to hypoglycaemia) are considered separately from those with normal hypoglycaemia awareness.

The aims of the present study were: (1) to assess the effects of acute hypoglycaemia on different memory processes (acquisition and recall) for conventional memory tasks; (2) to assess the effects of acute hypoglycaemia on a novel prospective memory task; and (3) to compare these effects in people with type 1 diabetes who had either normal or impaired hypoglycaemia awareness.

## Subjects and methods

The study protocol was approved by the Lothian Medical Research Ethics Committee, and all subjects gave informed consent to participation.

### Subjects

Subjects were required to have a diagnosis of type 1 diabetes, age between 18 and 45 years, a BMI between 20 and 30 kg/m$^2$, and $HbA_{1c}$ values between 7 and 10%. They were ineligible if they had any significant current medical condition or contraindication to experimental hypoglycaemia. Female patients were eligible only if a pregnancy test was negative.

Potential subjects were asked to grade their hypoglycaemia awareness on a scale from 1 to 7 [29], and their hypoglycaemia history was also discussed. People who chose a grade of 1 or 2 and reported no history of severe hypoglycaemia or significant change in their warning symptoms were categorised as having normal hypoglycaemia awareness (NHA). People who chose a grade between 3 and 7 and reported diminution of hypoglycaemic symptoms and episodes of unrecognised hypoglycaemia were categorised as having impaired hypoglycaemia awareness (IHA) [29]. People whose self-rated awareness appeared inconsistent with their hypoglycaemia history were ineligible, on the grounds that their awareness status was uncertain.

In total, 36 subjects were recruited (20 NHA, 16 IHA); their characteristics are given in Table 1. Microvascular complications were defined as any clinical diagnosis of diabetic retinopathy, neuropathy or nephropathy, the latter also requiring a urine albumin:creatinine ratio persistently above the local reference maximum or serum creatinine >150 μmol/l. The IHA group had significantly longer duration of diabetes ($t=3.937$, $df=34$, $p<0.001$) and more microvascular complications ($\chi^2=5.994$, $df=1$, $p=0.013$). Other comparisons were non-significant.

**Table 1** Subject characteristics

| | Hypoglycaemia awareness | |
|---|---|---|
| | Normal | Impaired |
| *n* | 20 | 16 |
| Male, female (*n*) | 12, 8 | 6, 10 |
| Age (years): median (range) | 29 (19–44) | 33.5 (22–43) |
| Diabetes duration (years): median (range) | 3.8 (1.1–20) | 15.5 (2–35) |
| $HbA_{1c}$ (%)[a] | 7.8±1.3 | 8.4±1.8 |
| BMI (kg/m$^2$) | 25.8±2.2 | 26.8±3.6 |
| Subjects with microvascular complications (%) | 1 (5) | 6 (38) |

Data are mean ± SD unless stated otherwise
[a] Non-diabetic reference range is 5.0–6.5%

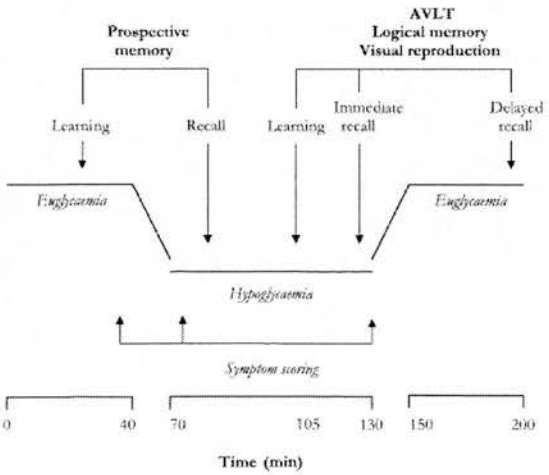


**Fig. 1** Study outline. The same timing was adhered to for euglycaemia studies

## Glucose clamp procedure

Each subject underwent one hypoglycaemic and one euglycaemic glucose clamp, separated by at least 2 weeks. Subjects were not informed of the order in which these occurred. On the evenings before studies, subjects took their normal insulin, fasted from 22.00 h (consuming only water) and omitted insulin on the morning of the study. Subjects were encouraged to avoid hypoglycaemia during the 48 h before the planned studies by reducing insulin doses if necessary. Studies were postponed if subjects detected any episode of hypoglycaemia by symptoms or routine blood testing during this period; this resulted in six postponements (two NHA, four IHA).

An antecubital vein and a dorsal hand vein were cannulated in the non-dominant arm for infusions and blood sampling. The hand was placed within a heated blanket to arterialise venous blood. Arterialised venous samples were drawn every 5 min for measurement of whole blood glucose (YSI 2300 STAT analyser, Yellow Springs, OH, USA). An infusion of soluble human insulin (Actrapid; NovoNordisk, Crawley, UK) was started at 1.5 mU kg$^{-1}$ min$^{-1}$, and 20% glucose solution was infused at a variable rate to achieve the desired blood glucose concentrations.

The blood glucose concentration was initially stabilised at 4.5 mmol/l (euglycaemia), and maintained for 40 min. During the hypoglycaemia studies, the blood glucose was then lowered over 20 min to 2.5 mmol/l and maintained for 60 min, then raised to 4.5 mmol/l and maintained for a further 75 min. Blood glucose was maintained at 4.5 mmol/l throughout the euglycaemia studies.

## Memory tests

Two parallel versions of each test were available, which were combined to give two batteries. The order in which subjects were exposed to these batteries and the hypoglycaemia–euglycaemia order were counterbalanced within the NHA and IHA groups. The study outline is given in Fig. 1.

*Prospective memory* This was a novel test, based on a method developed by Titov and Knight [21]. Two videos showing the view of a pedestrian journeying around central Edinburgh were created, and for each a 'shopping list' of 21 tasks was written on 21 cards. These were made up of 'buy' tasks (e.g. 'Buy tennis balls at A&B Sports'), 'do' tasks (e.g. 'Book a table at Smith's restaurant') and 'question' tasks (e.g. 'What is the advertised loan rate at the Mercantile Bank?'). Subjects were asked to read the cards twice at a normal reading pace, placing each card face down once it had been read. Subjects then watched the video and scored points for answering questions or stating actions at the relevant places. These were not brought to subjects' attention, and points were not awarded for answers given at inappropriate times. Pilot studies indicated that the two videos were of equivalent difficulty. Each lasted approximately 10 min, and were similar in terms of the number of shops passed (about 80). In the present study, subjects read the cards (learning) during initial euglycaemia, and recall was tested after approximately 40 min during the experimental period of induced hypoglycaemia/euglycaemia. Thus, the experimental manipulation examined the effects of hypoglycaemia on recall.

*Conventional memory tests* For the following memory tests, acquisition and immediate recall were tested during the experimental hour (euglycaemia or hypoglycaemia). Delayed recall was tested 90 min later during euglycaemia, without further exposure to the learned material. Thus, the

experimental manipulation here examined the effects of hypoglycaemia on acquisition (learning).

(1) Auditory Verbal Learning Test (AVLT)—immediate and delayed [30]. Fifteen words were read to the subject, who tried to recall the words immediately. This was repeated a further four times, and the immediate recall score was the sum of correct responses for the five trials. The delayed recall score was the number correctly recalled from a single trial.
(2) Logical Memory Test—immediate and delayed. In this subtest from the Wechsler Memory Scales—Revised [31], a short story was read to the subject, who tried to recount it immediately. Points were obtained for recollection of specific details and story themes.
(3) Visual Reproduction—immediate and delayed, from the Wechsler Memory Scales—Revised [31]. Each of five line drawings was shown to the subject for 10 s and then hidden, and the subject tried to reproduce the drawing from memory. Reproductions were scored according to strict criteria.

### Symptom scores

Subjects scored their symptoms of hypoglycaemia at baseline and during the experimental period using the Edinburgh Hypoglycaemia Scale [32].

### Statistical analysis

Scores on memory tasks were compared using repeated-measures ANOVA. Memory test score during euglycaemia versus hypoglycaemia was the repeated measure (within-subjects factor). Hypoglycaemia awareness, order of hypoglycaemia–euglycaemia and order of test battery were between-subjects factors. Statistical significance was accepted at $p<0.05$ and $\eta^2$ was used to indicate effect size. Analyses were performed using SPSS 11.0 (SPSS Inc., Chicago, IL, USA).

## Results

### Blood glucose

During the hypoglycaemic condition, mean±SD blood glucose was 2.5±0.2 mmol/l in the NHA group, and 2.5±0.2 mmol/l in the IHA group ($p=0.468$). During euglycaemia, mean blood glucose concentration was 4.5±0.2 mmol/l (NHA) and 4.5±0.3 mmol/l ($p=0.643$) (IHA).

### Symptoms

Total symptom scores did not change during euglycaemia. During the hypoglycaemic condition, mean symptom scores rose in both the NHA group (baseline 23.2±4.4 vs experimental 44.1±22.2; $p<0.001$) and the IHA group (22.9±7.0 vs 28.8±8.3; $p=0.001$). The increment in symptom scores was significantly greater in the NHA group (interaction between glycaemic condition and awareness status, $p=0.002$).

### Memory tasks

Results are given in Table 2. There were no significant effects of order of exposure to glycaemic condition or test battery.

*Comparison of the effect of hypoglycaemia in NHA and IHA subjects* The interaction between glycaemic state and hypoglycaemia awareness designation (hereafter referred to as the glycaemia×awareness interaction) was not significant for any tests. This means that there was no significant difference in the effects of hypoglycaemia on cognitive function between the NHA and IHA groups. In some cases, there was a statistically significant effect of hypoglycaemia in one group but not the other; however, in each case hypoglycaemia was associated with poorer performance in both groups, and achievement of statistical significance in one group but not the other may have been due to chance. In the absence of significant glycaemia × awareness interactions, the effect of hypoglycaemia on memory performance was determined for all subjects combined.

*Prospective memory* Recall was significantly impaired in all patients combined during hypoglycaemia ($\eta^2=0.255$, $p=0.004$). IHA subjects performed better overall than NHA subjects ($p=0.018$).

*Immediate verbal memory* Immediate recall was significantly impaired during hypoglycaemia for the AVLT ($\eta^2=0.268$, $p=0.003$) and logical memory test ($\eta^2=0.233$, $p=0.007$) in all subjects combined. The IHA group performed significantly better than the NHA group on the logical memory task ($p=0.030$).

*Delayed verbal memory* Delayed recall was significantly impaired during hypoglycaemia for both AVLT ($\eta^2=0.373$, $p<0.001$) and logical memory test ($\eta^2=0.315$, $p<0.001$) in all subjects combined. Again, the IHA group performed better overall on the logical memory task ($p=0.031$). Hypoglycaemia had no effect on the proportion of information retained (delayed score as a percentage of immediate score).

Table 2 Results of memory tests

| Test | NHA subjects alone | | | IHA subjects alone | | | All subjects | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Eu | Hypo | *p* value | Eu | Hypo | *p* value | Eu | Hypo | Glycaemia | | Awareness | | $G \times A$ interaction | | |
| | | | | | | | | | $\eta^2$ | *p* value | $\eta^2$ | *p* value | $\eta^2$ | *p* value |
| Prospective memory | | | | | | | | | | | | | | | |
| Immediate | 11.4±3.9 | 10.1±4.9 | 0.121 | 15.4±4.2 | 13.3±4.9 | 0.013 | 13.2±4.4 | 11.6±5.1 | 0.255 | 0.004 | 0.185 | 0.018 | 0.020 | 0.462 | |
| AVLT | | | | | | | | | | | | | | | |
| Immediate | 51.8±7.8 | 45.1±9.4 | 0.003 | 53.1±12.5 | 50.3±9.9 | 0.257 | 52.4±10.0 | 47.4±9.8 | 0.268 | 0.003 | 0.038 | 0.303 | 0.058 | 0.198 | |
| Delayed | 10.0±2.4 | 8.3±2.7 | 0.012 | 10.9±3.7 | 9.3±3.3 | 0.008 | 10.4±3.1 | 8.7±3.0 | 0.373 | 0.000 | 0.033 | 0.334 | 0.000 | 0.927 | |
| Retained (%) | 79.9±18.7 | 77.1±18.3 | 0.530 | 81.7±16.8 | 75.5±18.9 | 0.104 | 80.7±17.6 | 76.4±18.3 | 0.077 | 0.138 | 0.000 | 0.981 | 0.011 | 0.578 | |
| Logical memory | | | | | | | | | | | | | | | |
| Immediate | 15.6±3.5 | 13.1±3.3 | 0.011 | 17.3±3.9 | 16.3±4.7 | 0.223 | 16.4±3.7 | 14.5±4.3 | 0.233 | 0.007 | 0.157 | 0.030 | 0.053 | 0.221 | |
| Delayed | 13.1±3.7 | 11.0±3.4 | 0.010 | 15.8±4.5 | 13.8±4.3 | 0.047 | 14.3±4.2 | 12.2±4.0 | 0.315 | 0.001 | 0.155 | 0.031 | 0.001 | 0.898 | |
| Retained (%) | 84.2±16.8 | 83.4±16.3 | 0.903 | 89.6±11.2 | 84.2±14.4 | 0.223 | 86.6±14.6 | 83.8±15.3 | 0.022 | 0.431 | 0.027 | 0.385 | 0.013 | 0.548 | |
| Visual memory | | | | | | | | | | | | | | | |
| Immediate | 96.2±8.0 | 96.4±7.8 | 0.890 | 88.3±15.0 | 89.3±12.7 | 0.746 | 92.7±12.1 | 93.3±10.7 | 0.005 | 0.707 | 0.190 | 0.016 | 0.002 | 0.816 | |
| Delayed | 82.8±21.3 | 77.5±25.0 | 0.128 | 81.3±20.9 | 85.1±17.3 | 0.277 | 82.1±20.9 | 80.9±22.0 | 0.004 | 0.745 | 0.007 | 0.669 | 0.116 | 0.066 | |
| Retained (%) | 85.4±19.6 | 80.0±24.1 | 0.122 | 91.9±16.2 | 94.9±11.9 | 0.570 | 88.3±18.2 | 86.6±20.8 | 0.006 | 0.675 | 0.099 | 0.090 | 0.069 | 0.162 | |

Data are mean ± SD

Effect of glycaemic condition is shown first for NHA and IHA subjects, and then glycaemic condition, hypoglycaemia awareness and glycaemia × awareness ($G \times A$) interaction for all subjects combined

*Eu* euglycaemia, *Hypo* hypoglycaemia

*Visual memory* No significant effects of hypoglycaemia were seen. Mean euglycaemia scores of 96.2 (NHA) and 88.3 (IHA) from a maximum of 104 indicated marked ceiling effects. The NHA group scored better ($p=0.016$) on immediate visual memory.

## Discussion

Performance on the novel test of prospective memory was impaired significantly during the hypoglycaemia studies and, as learning always took place during euglycaemia, the results indicate that recall had been disrupted by hypoglycaemia. The effect size of hypoglycaemia on prospective memory scores ($\eta^2$) was similar to that for the conventional memory tests, which suggests that it is a similarly sensitive measure of hypoglycaemic memory dysfunction. The prospective memory task is also intuitively more reflective of memory in daily life, where list-learning is rarely relevant. In the paper upon which it was based, performance on the video task correlated well with performance on the same task in a real-life setting [21]. The data suggest that people with diabetes may suffer inability to act on previously made plans, and thus effectively to organise aspects of their daily life, as a consequence of hypoglycaemia.

Some limitations of the prospective memory task must be considered [21]. First, subjects are unable to explore the environment to obtain further cues if they suspect that an action is to be performed at a location, but cannot fully remember it. Second, they are unable to retrace their steps if an action is recalled after the location has been passed. Third, to make the task sufficiently difficult, more items are given than subjects would attempt to remember in real life. Fourth, subjects are unable to prioritise items and plan their journey. These points reflect the fact that in real life, prospective memory performance is not simply determined by accuracy of response to cues, but is also dependent on organisational and other strategies. Thus, the effect of hypoglycaemia on real-life prospective memory may be more or less than suggested by the present study, depending on whether these strategies are themselves sensitive or resistant to hypoglycaemia.

It is also possible that poorer performance during hypoglycaemia was caused by impairment of cognitive functions other than memory. The video required sustained attention for around 10 min, and attention has been shown to be impaired during hypoglycaemia [33, 34]. Deterioration of visual information processing may have reduced the subjects' ability to glean information from the video [11, 12]. Alternatively, the results may have resulted from a general effect on cognition, as very few cognitive abilities that have been tested during hypoglycaemia have not shown deterioration [2]. These distinctions may be neuropsychological rather than practical; whatever the mechanism, hypoglycaemia impaired the ability to act on information learned previously, which in everyday life would be perceived to be a memory problem.

Immediate recall performance for the two verbal memory tasks (AVLT and logical memory) was impaired significantly during hypoglycaemia, which is consistent with previous studies [15, 16]. Delayed recall was always tested during euglycaemia, and it can be inferred that the significant impairment in delayed recall following hypoglycaemia resulted from a hypoglycaemic effect on learning and/or consolidation. A 'hangover' effect of hypoglycaemia is unlikely to be responsible, as delayed recall was tested 50 min after restoration of euglycaemia. The only robust study to show residual cognitive impairment demonstrated this 20 min after hypoglycaemia [35]; earlier studies reported impaired cognitive function 45–90 min after hypoglycaemia [8, 36–40], but each had a methodological deficiency, such as failure to test statistical significance or the absence of a euglycaemia control arm.

Correction of hypoglycaemia took approximately 20 min, the limiting factors being the continuing insulin infusion and the maximum infusion rate of hypertonic glucose solution. The possibility that hypoglycaemia impaired performance through an effect on consolidation in the immediate postlearning period cannot be excluded. However, on this basis it is also relatively unimportant to separate learning and immediate consolidation, as hypoglycaemia in the daily life of a person with diabetes will not be reversed more quickly. The practical conclusion that may be drawn from the verbal memory results is that hypoglycaemia impairs the learning of information so that it may be recalled at a later time. For example, if hypoglycaemia occurred while studying, the student would be advised to review material that had recently been examined.

Visual memory was not impaired significantly by hypoglycaemia, but with mean scores of 93 and 82% for immediate and delayed recall, respectively, during euglycaemia, a ceiling effect was evident. Future work on this aspect of memory should employ tasks with a larger range of items, especially more difficult items.

There was no evidence of a different effect of hypoglycaemia in IHA and NHA subjects. This may have been related to inadequate statistical power for this specific between-subject analysis. However, with a total of 36 subjects and a repeated-measures design, the study was large by comparison with others in this research area. There are some limitations of the between-group comparisons. First, we cannot exclude overlap, even though our method of assessing awareness has been shown to be a good predictor of severe hypoglycaemia at the group level [29]. Subjects

with similar levels of awareness could receive different classifications as a result of answering with confidence or caution. Second, the two groups were not well matched for other characteristics, which may have confounded the effect of hypoglycaemia on memory performance, particularly the duration of diabetes, the presence of microvascular complications and overall memory performance. For these reasons, the present study cannot exclude a potential difference between people with IHA and NHA.

An important consideration is that studies of this type, which compare group means using a powerful within-subject design, do not have much power to examine individual differences in the effects of hypoglycaemia on cognition. There is, in our experience, consistent anecdotal evidence from both clinical practice and research to suggest that some individuals are much more susceptible to hypoglycaemia-induced cognitive dysfunction than others. To assess this experimentally is impracticable: for this type of intensive study, an uncommonly large number of subjects would be required. However, given the possibility of inter-individual differences, practical recommendations to people with diabetes should not be too rigid.

In conclusion, the present study has confirmed that there is a disruptive effect of hypoglycaemia on both the specific processes of learning and recall, which is relevant to the behaviour of people with diabetes during daily life. The prospective memory test appears to be a sensitive measure of memory dysfunction during hypoglycaemia that may have greater ecological validity than conventional memory tests, and lends weight to the use of laboratory data to derive practical recommendations for people with diabetes.

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BRIEF REPORT

# Acute Hypoglycemia Impairs Nonverbal Intelligence

## Importance of avoiding ceiling effects in cognitive function testing

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Acute hypoglycemia causes a progressive, reversible deterioration in cognitive function that becomes detectable at blood glucose concentrations below ~3.0–3.4 mmol/l (1). In an earlier study in nondiabetic subjects, we reported that several facets of attention deteriorated significantly at an arterialized blood glucose level of 2.6 mmol/l (2). However, performance on Raven's Progressive Matrices (RPM) was not significantly impaired at either 20 min or completion of the test (with no time limit). This was unexpected, because, previously, various domains of cognitive function had consistently been impaired at this level of hypoglycemia (1). Additionally, the RPM is acknowledged as being among the best indicators of general fluid intelligence (3) and has a substantial correlation with working memory (4), which is exquisitely sensitive to hypoglycemia (5).

Hypoglycemia disrupts performance on different cognitive function tests to a variable degree, leading to speculation that different mental functions vary in their sensitivity to hypoglycemia. This is complicated by a lack of a universally accepted battery of cognitive measures and variable experimental methodology (6,7). We speculated that the higher-level cognitive skills required for abstract problem solving (as in the RPM) are resistant to the effects of hypoglycemia. However, this contradicted a widely held opinion that higher-level skills are more sensitive to hypoglycemia than simple, repetitive cognitive or motor tasks (1). An alternative explanation was the possibility of a ceiling effect, as the RPM includes a large proportion of easy problems that involve straightforward pattern completion, and mean scores during euglycemia and hypoglycemia were 49.5 and 48.7, respectively, out of a maximum of 60. The present study was designed to test the ceiling effect hypothesis by substituting two more difficult tests of general fluid intelligence that are known to be discriminatory in highly able adults. Raven's Advanced Progressive Matrices (RAPM) (8) uses harder problems than RPM, which consist of geometric designs from which the subject must induce logical rules, hold the rules in working memory, and apply them simultaneously to complete a pattern (9). The Alice Heim 5 test (AH5) similarly requires identification and application of simultaneous patterns to complete verbal, numerical, and geometric sequences (10).

**RESEARCH DESIGN AND METHODS**— Sixteen nondiabetic volunteers were studied. Their mean score on the National Adult Reading Test was 40, which approximates to a Wechsler Adult Intelligence Scale-Revised full-scale IQ score of 118. Each subject underwent two hyperinsulinemic glucose clamps, with experimental states of euglycemia (arterialized blood glucose 4.5 mmol/l) and hypoglycemia (2.5 mmol/l), during which subjects completed the RAPM and AH5. Parallel versions were created by separating odd- and even-numbered items, with sets 1 and 2 of the RAPM being combined. Both the RAPM and AH5 were scored after 20 min, with maximum possible scores of 36 for AH5 and 24 for RAPM. Subjects also completed the Trail Making B (TMB) and Digit Symbol Substitution (DSST) tests. The order of euglycemia-hypoglycemia and cognitive test battery was counterbalanced and included as a between-subject factor in repeated-measures ANOVA.

**RESULTS**— The scores are given in Table 1 as means (±SD), except TMB, which is given as completion time in seconds. Performance on TMB, DSST, and RAPM deteriorated significantly during hypoglycemia, with a trend toward deterioration on AH5. The effect size of hypoglycemia on RAPM was substantial, amounting to approximately three-quarters of an SD.

**CONCLUSIONS**— These results suggest that our earlier findings were indeed due to a ceiling effect (2) and indicate that hypoglycemia does impair general fluid intelligence, but it is necessary to use a test appropriate to the ability level of the participants. Research volunteers tend to be more able than the population they are intended to represent, and the ceiling effect may be common in studies of hypoglycemia. Care should be taken to avoid the ceiling effect, and, while general consensus on cognitive testing is desirable, a single battery of cognitive tests for all subjects may be inappropriate (7).

The different results for RAPM and AH5 may reflect differences in the standard administration of these tests: paper working is permitted for the AH5 but not for the RAPM. Holding and applying rules entirely in working memory is demanding (8), and as we have previously shown that working memory is obliterated by

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Abbreviations: AH5, Alice Heim 5 test; DSST, Digit Symbol Substitution; RAPM, Raven's Advanced Progressive Matrices; RPM, Raven's Progressive Matrices; TMB, Trail Making B.

**Table 1** —*Cognitive function scores during euglycemia and hypoglycemia, plus significance level and effect size for comparison*

| Test | Euglycemia | Hypoglycemia | *P* | $\eta^2$ |
|---|---|---|---|---|
| RAPM | 18.9 ± 3.1 | 16.5 ± 3.5 | 0.007 | 0.465 |
| AH5 | 15.6 ± 5.6 | 13.9 ± 5.0 | 0.057 | 0.269 |
| TMB | 38.8 ± 8.2 | 46.3 ± 16.1 | 0.037 | 0.339 |
| DSST | 87.7 ± 12.5 | 79.4 ± 9.3 | 0.019 | 0.409 |

Data are means ± SD.

moderate hypoglycemia (5), it is likely that this had a greater impact on RAPM than AH5.

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RESEARCH ARTICLE

# The symptoms of hyperglycaemia in people with insulin-treated diabetes: classification using principal components analysis

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## Abstract

**Background and aims** People with insulin-treated diabetes commonly experience symptoms of hyperglycaemia, but the nature of these symptoms and their origins are poorly understood. The aims of this study were (1) to identify and classify the symptoms of hyperglycaemia experienced by people with insulin-treated diabetes and (2) to identify patient characteristics associated with intensity of, and glycaemic threshold for, glycaemic symptoms.

**Methods** Common hyperglycaemic symptoms were identified from preliminary interviews. Eighteen symptoms were used in a questionnaire. Four hundred participants estimated the intensities with which they experienced these symptoms during hyperglycaemia. Principal components analysis (PCA) was used to examine correlations between symptoms. Associations between symptom intensity, glycaemic threshold, and other characteristics were examined with multiple regression.

**Results** In total, 361 participants (90.2%) reported experiencing hyperglycaemic symptoms. PCA suggested four symptom groupings: (1) feeling tense, irritability, restlessness, poor concentration (*agitation*) (2) thirst, dry mouth, need to urinate, not feeling right, sweet/funny taste, weakness (*osmotic*) (3) dizziness, blurred vision, light-headedness, weakness (*neurological*) (4) headache, nausea (*malaise*). Mean symptom intensity was associated with younger age. The median (range) estimated blood glucose threshold for symptom onset was 15 (8–30) mmol/L; there was a weak tendency for this threshold to be elevated in people who had impaired hypoglycaemia awareness.

**Conclusions** People with insulin-treated diabetes commonly reported symptoms associated with hyperglycaemia. PCA separated these into four groups. Osmotic symptoms appear to be specific to hyperglycaemia; symptoms in the other groups may suggest underlying physiological mechanisms, but are relatively non-specific. Symptoms are more intense in younger people and may be reported at lower blood glucose concentrations in people with normal awareness of hypoglycaemia. Copyright © 2003 John Wiley & Sons, Ltd.

**Keywords** diabetes; insulin; symptoms; hyperglycaemia; hypoglycaemia; factor analysis

## Introduction

People with diabetes are commonly exposed to fluctuations in blood glucose into hypo- and hyperglycaemic ranges. The symptoms of hypoglycaemia have

been described in detail, and their generation has been studied using different approaches [1]. These include experimentally induced hypoglycaemia with the use of different forms of pharmacological blockade [2,3], and statistical analysis of symptoms that had either been described during experimental hypoglycaemia [4] or derived from population surveys [5,6]. The statistical techniques of principal components analysis (PCA) and confirmatory factor analysis have been used to identify discrete groups, or domains, of symptoms, which correspond to underlying physiological mechanisms, and in adults include *neuroglycopenic*, *autonomic*, and *malaise* groups [1,6].

Symptoms of hyperglycaemia are often present at the time of diagnosis of diabetes, and the classical osmotic symptoms include thirst, polyuria, and nocturia [7]. However, there are few experimental studies of the symptoms of hyperglycaemia. When acute hyperglycaemia was induced by a hyperinsulinaemic glucose clamp technique, only one symptom (the need to urinate) was positively associated with this state [8]. However, hyperglycaemia induced by this method may not reflect everyday experience. In another study, many subjects reported symptoms that correlated positively with blood glucose concentrations [9], but these symptoms varied widely between subjects and subsequently were found to be unstable over time [10]. A study of all symptoms in type 2 diabetes listed thirst, dry mouth, polyuria, and polydipsia as symptoms of hyperglycaemia, but this study did not focus specifically on acute hyperglycaemia [11].

In our experience, people with insulin-treated diabetes recognize various unpleasant symptoms that are associated with transient hyperglycaemia, which may prompt them to measure blood glucose and modify their treatment. The perception of the development of hyperglycaemic symptoms may assist the individual to improve glycaemic control, and the early treatment of elevated blood glucose may prevent progression to metabolic decompensation. The principal aims of the present study were to determine which symptoms of hyperglycaemia are commonly reported by people with insulin-treated diabetes, and to ascertain whether any underlying structure could be identified to assist with the classification of the symptoms. Secondary aims were to determine whether the intensity of, and glycaemic threshold for, symptoms of hyperglycaemia are related to the coexisting state of hypoglycaemia awareness, the quality of glycaemic control, the duration of insulin therapy, and concomitant drug therapy.

## Methods

### Pilot study

A pilot study was performed to identify the possible symptoms of hyperglycaemia. Seventy patients with insulin-treated diabetes were asked to report, in an open-ended manner, symptoms that they associated with a high blood glucose. Their reports were grouped together as symptoms when identical wording was used or when it was clear that the same sensation was being described. A total of 25 different symptoms were reported, 17 of which were reported by more than one person (Table 1). In formulating the symptom questionnaire, one additional symptom (an increase in salivation) that had been reported by more than one subject in a previous study [9] was incorporated. These 18 symptoms were used to compile the symptom questionnaire for the main study. Patients participating in the pilot study did not take part in the main study.

### Subjects

To provide a robust solution using principal components analysis, the most cautious estimate of the number of subjects required is 10 times the number of variables under examination [12]. With 18 symptoms in the questionnaire, 180 subjects should suffice. A target of 400 subjects was set to provide a large safety margin.

Approval for the study was granted by the local research ethics committee. People were recruited during their routine visits at diabetes outpatient clinics in the Department of Diabetes at the Royal Infirmary of Edinburgh. Patients were eligible if they had received full insulin therapy for at least one year, had performed self-monitoring of blood glucose (irrespective of frequency), and were capable, in the opinion of the investigator, of answering the questionnaire. Patients using insulin at bedtime only were excluded, but any full-time regimen (twice daily injections or more often) was permitted. All consecutive patients who met the inclusion criteria and who consented to take part were recruited until the target of 400 was achieved. Characteristics of the participants are shown in Table 2. Written informed consent was obtained before the questionnaire was applied.

**Table 1. Symptoms associated with hyperglycaemia reported by more than one individual in pilot study**

| |
|---|
| Blurred vision |
| Thirst |
| Nausea |
| Not feeling right |
| Weakness |
| Dry mouth |
| Irritability |
| Light-headedness |
| Tiredness |
| Need to urinate |
| Headache |
| Restlessness |
| Sweet or funny taste |
| Stomach ache |
| Poor concentration |
| Dizziness |
| Feeling tense |

Table 2. Characteristics of insulin-treated diabetic subjects completing symptom questionnaire

| | Total | Type 1 | Type 2 |
|---|---|---|---|
| *n* | 400 | 288 | 112 |
| Male (%) | 229 (57.3) | 173 (60.1) | 57 (50.9) |
| Median age, in years (range) | 49 (18–84) | 41 (18–79) | 66 (35–84) |
| Median duration of diabetes, in years (range) | 14 (1–59) | 15 (1–59) | 12 (4–36) |
| Median duration of insulin therapy, in years (range) | 11 (1–59) | 15 (1–59) | 4 (1–33) |
| Mean $HbA_{1c}$ (SD) (%) | 8.6 (1.3) | 8.6 (1.4) | 8.7 (1.3) |

## Questionnaire

Each subject completed the questionnaire once. Subjects provided estimates, on the basis of prior experience, of the intensity with which they usually felt each symptom during periods of hyperglycaemia ('when your blood glucose level is high'). Hyperglycaemia was not defined more explicitly in terms of blood glucose concentration. Estimates of symptom intensity were recorded on a visual scale from 1 ('not at all') to 7 ('very strongly'). The participants were asked to indicate the approximate level of blood glucose at which they would expect to experience hyperglycaemic symptoms, on the basis of previous blood glucose testing, and to describe any other symptoms that they associated with hyperglycaemia. In addition, they were asked to rate their symptomatic awareness of hypoglycaemia on a visual scale from 1 (always aware) to 7 (never aware) [13]. Duration of diabetes and duration of insulin therapy, details of concomitant drug therapy, and most recent glycated haemoglobin ($HbA_{1c}$) measurements were recorded. Glycated haemoglobin was measured by high-performance liquid chromatography (BioRad Variant 2, Hemel Hempstead, UK; local non-diabetic range 5.0–6.5%).

## Principal components analysis

Data were analysed using SPSS 11.0 (SPSS, Chicago, IL). Correlations among the intensity ratings of the 18 symptoms were examined using principal components analysis. We refer the interested reader to the monograph by Child [12] for an accessible explanation of this technique. Briefly, this procedure examines the correlations among measured variables to determine whether there are groups of variables that occur together. This could be done informally: it might, for example, be apparent from inspection of correlation coefficients that there were three groups of symptoms, such that symptoms were strongly correlated with other symptoms in the same group but only weakly correlated with symptoms in other groups. In this hypothetical case, the entire data set could be reduced to three 'factors', each representing a group of variables from the original data set. If the within-group correlations had been high, then this process would greatly simplify the data set with only minimal loss of information. The value of this technique in examining symptoms arises from the fact that symptom grouping may hint at underlying physiological processes. For example, in previous studies, the symptoms of confusion, odd behaviour, inability to concentrate, drowsiness, and difficulty in speaking were highly correlated and were interpreted as the effects of neuroglycopenia; whereas hunger, sweating, trembling, anxiety, and a pounding heart formed a separate, highly correlated group, which was interpreted as the effects of autonomic activation [4–6].

Principal components analysis (PCA), sometimes referred to as *factor analysis*, is a statistical tool for extracting components that simplify the data, while retaining as much information as possible. Technically, *components* are computed first and then undergo a mathematical transformation called *rotation* to yield more easily interpretable 'factors'. Although there is a technical difference between 'component' and 'factor', for the sake of clarity, readers may consider *component* and *factor* to be interchangeable here. The *variance* explained by a factor indicates the amount of information from the original data that is contained within the computed factor. PCA initially generates the same number of components as the number of symptoms under examination, but most explain very little variance. Only components that usefully simplify the data are considered significant, and these are determined by selection of those that are outliers on a plot of variance (the scree-slope criterion) and/or those that explain more variance than a single variable from the original data (the 'eigenvalues greater than one' criterion).

PCA factors are mathematical constructs and their interpretation relies on *factor loadings*, which are the correlations between the original symptom scores and the extracted factors. Factors are considered to represent those symptoms with which they have moderate to high correlations. A cutoff for the correlation coefficient of 0.3 to 0.4 is generally applied [12], although this remains somewhat arbitrary. The internal consistency of the grouping of symptoms suggested by this method may be confirmed by computing Cronbach's Alpha statistic.

## Other statistical methods

Mean symptom intensity scores for each subject were calculated as the arithmetical means of the scores for each variable. Associations between these scores and other characteristics of the participants were investigated with Spearman rank correlations, Mann–Whitney *U* tests, and linear regression techniques. Scatter plots were examined for correlations to exclude artefacts from outlying cases.

# Results

## Symptom endorsements

Of the 400 respondents, 361 (90.2%) identified one or more of the 18 symptoms as associated with hyperglycaemia. The five most commonly endorsed symptoms

were 'thirst', 'dry mouth', 'not feeling right', 'need to urinate', and 'tiredness'. The absolute endorsement frequencies (i.e. any response greater than 'not at all') and the mean scores [1–7] for each symptom are given in Table 3.

## Principal components analysis

Two of the 18 symptoms ('stomach ache' and 'increased salivation') had absolute endorsement levels below 20%. Weakly endorsed variables cannot be reliably interpreted by PCA, and these symptoms were excluded from subsequent analysis. PCA was performed on symptoms' data from the 361 subjects, who reported any hyperglycaemic symptoms, using the remaining 16 symptoms. The first unrotated principal component has strong loadings (0.47 or greater) from all symptoms (Table 4) and accounts for 37.7% of the total variance. The high loadings and high internal reliability (Cronbach's Alpha 0.89) indicate that it is a general hyperglycaemic symptoms' component. Because of this, rotation was performed using the Direct Oblimin method, which permits factors to be correlated (non-orthogonal).

A four-factor solution was appropriate, based on both eigenvalues greater than one and scree-slope inspection. Variances accounted for by each factor and loadings from each of the 16 symptoms are shown in Table 4. Factor loadings greater than 0.35 were considered significant. The first factor ('feeling tense', 'irritability', 'restlessness', and 'poor concentration') contains symptoms that appear to have in common some aspect of *mental agitation*. The second factor ('thirst', 'dry mouth', 'need to urinate', 'not feeling right', 'sweet or funny taste', and 'weakness') mainly represents symptoms resulting from the *osmotic* effects of hyperglycaemia. The third factor ('dizziness', 'blurred vision', 'light-headedness', and 'weakness') contains symptoms similar to those designated *neuroglycopenic* in previous studies of hypoglycaemic symptoms using factor analysis [4–6], although *neurological* may be a better nomenclature in the present context. The fourth factor has strong loadings from 'headache', 'nausea', and 'sweet or funny taste'. Headache and nausea also appeared as a factor (labelled *malaise*) in hypoglycaemia studies [4–6].

The total variance explained by the four-factor model is 62.9%. The factors are correlated, and so the sum of the

**Table 3. Mean intensity scores and absolute endorsement rates for each symptom**

| Symptom | Mean score (SD) | Absolute endorsement (%) |
|---|---|---|
| Thirst | 4.06 (2.05) | 79.5 |
| Dry mouth | 4.09 (2.09) | 78.7 |
| Not feeling right | 3.80 (2.08) | 75.6 |
| Need to urinate | 3.71 (2.35) | 64.5 |
| Tiredness | 3.30 (2.23) | 61.5 |
| Irritability | 2.80 (2.18) | 48.2 |
| Poor concentration | 2.59 (1.96) | 47.9 |
| Weakness | 2.36 (1.85) | 43.2 |
| Sweet/funny taste | 2.53 (2.09) | 41.0 |
| Restlessness | 2.30 (1.88) | 40.2 |
| Feeling tense | 2.19 (1.82) | 38.0 |
| Headache | 2.12 (1.81) | 34.1 |
| Blurred vision | 1.81 (1.45) | 29.4 |
| Light headed | 1.80 (1.53) | 26.9 |
| Nausea | 1.80 (1.54) | 26.6 |
| Dizziness | 1.70 (1.50) | 22.4 |
| Stomach ache | 1.42 (1.17) | 13.9 |
| Increased saliva | 1.34 (1.07) | 11.9 |

**Table 4. Results of principal components analysis of symptom intensity scores, with Direct Oblimin Rotation**

| | First unrotated principal component | Rotated factors | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| Variance (%) | 33.7 | 24.6 | 21.5 | 21.2 | 13.3 |
| Cronbach alpha | 0.86 | 0.79 | 0.79 | 0.74 | 0.51 |
| Symptom | Component loadings | Pattern matrix loadings | | | |
| Thirst | 0.51 | −0.13 | **−0.89** | 0.03 | −0.03 |
| Dry mouth | 0.57 | −0.10 | **−0.82** | 0.03 | 0.14 |
| Not feeling right | 0.70 | 0.01 | **−0.41** | 0.14 | 0.15 |
| Need to urinate | 0.44 | 0.08 | **0.73** | −0.16 | 0.05 |
| Tiredness | 0.62 | **0.36** | 0.20 | 0.33 | −0.07 |
| Irritability | 0.65 | **0.80** | 0.05 | −0.04 | 0.08 |
| Poor concentr'n | 0.67 | **0.56** | 0.09 | 0.29 | −0.07 |
| Weakness | 0.61 | 0.18 | **0.40** | **0.47** | −0.26 |
| Sweet/odd taste | 0.57 | 0.14 | **0.42** | −0.06 | **0.43** |
| Restlessness | 0.63 | **0.78** | −0.03 | −0.06 | 0.13 |
| Feeling tense | 0.58 | **0.87** | −0.11 | −0.03 | −0.04 |
| Headache | 0.51 | 0.08 | 0.05 | 0.07 | **0.73** |
| Blurred vision | 0.45 | −0.09 | −0.06 | **0.76** | 0.08 |
| Light-headedness | 0.62 | 0.08 | −0.03 | **0.73** | 0.14 |
| Nausea | 0.48 | 0.01 | 0.02 | 0.17 | **0.69** |
| Dizziness | 0.59 | 0.03 | −0.06 | **0.83** | 0.07 |

Factor loadings greater than 0.35 are highlighted.
concentr'n, concentration.

individual variances exceeds this total. Cronbach's Alpha was calculated as a measure of internal consistency for the general hyperglycaemia factor and each of the four rotated factors (Table 4). All except the malaise factor had high internal consistency coefficients (>0.7).

## Sub-group analyses

Because of the large number of participants, it was possible to repeat the PCA using sub-groups. For comparison based on glycaemic control, the original data were divided into two groups of participants with $HbA_{1c}$ above and below the median. For comparison based on hypoglycaemia awareness, the data were divided into those participants who gave a rating of 1 or 2 (normal awareness) and those who gave a rating of 3 to 7 (impaired awareness) [13]. In each sub-group analysis, essentially the same symptoms loaded onto the same four factors as in the analysis of the full data set. Results of sub-group analyses are not shown.

## Factors associated with symptom intensity and estimated glycaemic threshold

A mean symptom intensity score was calculated for each participant as the mean of scores for the 16 symptoms. Spearman rank correlations were calculated between this score and the following independent variables: age, duration of insulin therapy, hypoglycaemia awareness rating, and current $HbA_{1c}$. The full data set ($n = 400$) was used; participants reporting no symptoms had the minimum mean score of 1. A strong negative association with age ($\rho = -0.437$, $p < 0.001$) and a small positive association with $HbA_{1c}$ ($\rho = 0.149$, $p = 0.003$) were observed. Mann–Whitney $U$ tests were used to assess the effects of the binary variables sex, type of diabetes, and use of beta-adrenoceptor blocking drugs. Subjects' gender and use of beta-blockers were not associated with significant differences in mean symptom score. Subjects with type 1 diabetes had higher median reported symptom intensities than subjects with type 2 diabetes (2.41 vs 1.82, 2-tailed $z = -3.867$, $p < 0.001$).

A total of 340 subjects (85%) were able to estimate a glycaemic threshold for the onset of symptoms of hyperglycaemia. The mean (range) estimated threshold was 15.1 (8–30) mmol/L. The estimated threshold was examined for associations with the same independent variables. Significant correlations were observed for duration of insulin therapy ($\rho = 0.157$, $p = 0.004$) and hypoglycaemia awareness ($\rho = 0.204$, $p < 0.001$). No significant associations were observed for age, $HbA_{1c}$, sex, type of diabetes, or use of beta-blockers.

Multiple regression models were constructed, with mean symptom score and estimated glycaemic threshold separately entered as the dependent variables. The seven independent variables used above were entered in each model, with stepwise removal of non-significant variables. Results are given in Table 5. With mean symptom intensity as the independent variable, there was a significant negative association with age and a statistically significant but small positive association with $HbA_{1c}$; the total $R^2$ for this model was 0.197. With estimated glycaemic threshold as the independent variable, there were statistically significant but small associations with hypoglycaemia awareness, age, $HbA_{1c}$, and sex; however, the variance explained by this model was very small (total $R^2 = 0.118$).

## Other symptoms of hyperglycaemia

With open-ended questioning, 68 (17%) subjects reported hyperglycaemic symptoms other than the 18 included in the questionnaire. The commonest were sweating (15 subjects), muscular ache (14 subjects), a flushed or warm sensation (14 subjects), and heavy or stiff limbs (10 subjects). Various other symptoms were each reported by fewer than five subjects.

# Discussion

In the present study, 90% of adults with insulin-treated diabetes reported experiencing symptoms associated with hyperglycaemia. When symptoms were examined using PCA, it was observed that the co-variation between intensity scores for the 16 most commonly reported symptoms could be accounted for by 4 factors. On the

**Table 5. Results of multiple regression analyses**

| Dependent variable | Independent variable | $R^2$ change (total $R^2$) | Standardized beta | $p$ |
|---|---|---|---|---|
| Mean score for 16 principal symptoms | Age | 0.182 | −0.410 | <0.001 |
| | $HbA_{1c}$ | 0.015 (0.197) | 0.125 | 0.007 |
| Blood glucose threshold for onset of symptoms | Hypoglycaemia awareness | 0.062 | 0.259 | <0.001 |
| | Age | 0.020 | 0.160 | 0.003 |
| | $HbA_{1c}$ | 0.018 | 0.154 | 0.005 |
| | Sex (0 = male, 1 = female) | 0.018 (0.118) | −0.137 | 0.012 |

Only associations yielding $p < 0.05$ are shown.

basis of the symptoms associated with each factor, they have been labelled here as *neurological*, *osmotic*, *mental agitation*, and *malaise*.

One of the most striking aspects of these results is the similarity between symptoms of hyperglycaemia and hypoglycaemia. Two of the four factors are similar to factors that were labelled 'neuroglycopenic' and 'malaise' when derived from hypoglycaemic symptoms. The first hyperglycaemia factor, apparently representing mental agitation, includes poor concentration (also one of the neuroglycopenic symptoms of hypoglycaemia), and irritability and restlessness, which are described during hypoglycaemia in children [14,15]. Clearly, neuroglycopenia is unlikely to be the cause of symptoms in hyperglycaemia, but its generation may have a neurological basis by the direct effect of hyperglycaemia on cerebral function. However, clinical experience relates that these symptoms may also be reported by people who are unwell with various illnesses. It may be that these are non-specific symptoms associated with systemic upset of any sort, segregated by factor analysis into groups representing physical and mental malaise.

Osmotic symptoms were the most strongly endorsed symptoms of hyperglycaemia in this study. Symptoms of thirst, dry mouth, and need to urinate have not been associated with hypoglycaemia, and these symptoms appear to be specific for hyperglycaemia. The autonomic symptoms of hypoglycaemia (sweating, palpitations, shaking, and hunger) did not also appear as hyperglycaemic symptoms in this study. It would appear, therefore, that the two states may be best distinguished by the presence of osmotic or autonomic symptoms, whereas a variety of mental and physical symptoms may be common to both. Incidentally, it was noted that many subjects reported that symptoms of tiredness and aching muscles were more common during early hyperglycaemia, whereas thirst and increased urination emerged with more prolonged or severe hyperglycaemia. It is plausible that osmotic symptoms would be delayed, as they may result in part from dehydration due to increased urine formation. Participants were not formally asked about symptoms at different levels or durations of hyperglycaemia, and so the study design did not permit assessment of this putative association. However, an absence of osmotic symptoms in early hyperglycaemia would be expected to make identification of this state more difficult. The ability to distinguish between hypo- and hyperglycaemia could be further impaired in people whose autonomic symptoms of hypoglycaemia are diminished in association with long duration of diabetes or frequent exposure to hypoglycaemia [1]. Previous studies have confirmed that estimation of blood glucose from symptoms is unreliable in many patients [8,16,17].

The symptoms of hyperglycaemia identified in this study are similar to those reported in other studies [8,9,11]. Some subjects also reported symptoms of hyperglycaemia other than those included in the questionnaire, most commonly sweating, myalgia, a flushed or warm sensation, and heavy or stiff limbs. These symptoms were reported rarely, although it is possible that more frequent endorsement would have resulted from prompted questions. Future studies should include these symptoms.

Regression analyses were used to examine the influence of various subject characteristics on mean symptom intensity and the estimated glycaemic threshold for the onset of symptoms. Age was modestly associated with mean symptom intensity, with older respondents reporting less intense symptoms. The estimated glycaemic threshold was weakly associated with hypoglycaemia awareness, such that people with diminished awareness of hypoglycaemia tended to report higher glycaemic thresholds for hyperglycaemic symptoms. This association may be explained by tolerance to both hypo- and hyperglycaemic extremes in individuals with erratic blood glucose control, or by a defect in glucose sensing leading to a single state of impaired glycaemic awareness. The variances explained by these models were modest, indicating substantial individual variation and limiting the reliability of associations.

Further research in this area should include studies to determine the threshold for hyperglycaemic symptoms under experimental conditions, with comparisons between diabetic people with normal and impaired hypoglycaemia awareness, and strict and poor glycaemic control. It would also be pertinent to determine how soon symptoms develop after the onset of hyperglycaemia, whether the duration and degree of hyperglycaemia affect the nature and correlational structure of the symptoms experienced, and whether the four groups of symptoms identified in the present study are differentially affected.

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